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=> s "EtxB"
L1 267 "ETXB"

=> s l1 and allergy
L2 1 L1 AND ALLERGY

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L2 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
1999:451202 Document No. 131:82960 **EtxB** or ganglioside GM1 for treating allergic or hypersensitivity conditions. Williams, Neil Andrew; Hirst, Timothy Raymond; Bienenstock, John (Oratol Limited, UK). PCT Int. Appl. WO 9934817 A1 19990715, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB70 19990108. PRIORITY: GB 1998-487 19980109.
AB The use of an agent in the manufacture of a medicament to treat an allergic condition and/or a hypersensitivity condition is described. The agent is capable of modulating a ganglioside-associated activity. The agent is not coupled to an antigen. The modulation of the ganglioside-associated activity affects an allergic condition and/or a hypersensitivity condition. Examples of such modulators include ganglioside GM1 and E. coli enterotoxin B subunit.

=> s l1 and treatment
L3 25 L1 AND TREATMENT

=> dup remove l3
PROCESSING COMPLETED FOR L3
L4 6 DUP REMOVE L3 (19 DUPLICATES REMOVED)

=> d l4 1-6 cbib abs

L4 ANSWER 1 OF 6 MEDLINE on STN DUPLICATE 1
2004067873. PubMed ID: 14769038. The disassembly and reassembly of mutants of Escherichia coli heat-labile enterotoxin: replacement of proline 93 does not abolish the reassembly-competent and reassembly-incompetent states. Cheesman C; Freedman R B; Ruddock L W. (Department of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK.) Biochemistry, (2004 Feb 17) 43 (6) 1618-25. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.
AB The carrier moiety of heat-labile enterotoxin of Escherichia coli (**EtxB**) is formed by the noncovalent association of identical monomeric subunits, which assemble, in vivo and in vitro, into exceptionally stable pentameric complexes. In vitro, acid disassembly followed by neutralization results in reassembly yields of between 20% and 60% depending on the identity of the salts present during the acid denaturation process. Loss of reassembly competence has been attributed to isomerization of the native cis-proline residue at position 93. To characterize this phenomenon further, two mutants of **EtxB** at

proline 93 (P93G and P93A) were generated and purified. The proline variants reveal only minor differences in their biophysical and biochemical properties relative to wild-type protein, but major changes were observed in the kinetics of pentamer disassembly and reassembly. Additionally, a loss of assembly competence was observed following longer term acid **treatment**, which was even more marked than that of the wild-type protein. We present evidence that the loss of assembly competence of these mutants is best explained by a cis/trans peptidyl isomerization of the unfolded mutant subunits in acid conditions; this limited reassembly competence and the biophysical properties of the native P93 mutant pentamers imply the retention of the native cis conformation in the nonproline peptide bond between residues 92 and 93 in the mutated proteins.

L4 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
2003:6139 Document No. 138:68275 Mutant forms of enterotoxin (**EtxB**) and cholera toxin (CtxB), and their therapeutic uses as target site-specific carriers. Hirst, Timothy Raymond (University of Bristol, UK). PCT Int. Appl. WO 2003000899 A1 20030103, 84 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB2829 20020620. PRIORITY: GB 2001-15382 20010622.

AB The present invention describes the use of a mutant form of enterotoxin subunit B (**EtxB**) or cholera toxin subunit B (CtxB) to deliver an agent to a target cell wherein the mutant has GM-1 binding activity, and a reduced immunogenic and immunomodulatory activity relative to the wild type form of **EtxB** or CtxB. Specifically, the mutant CtxB with His to Ala substitution at position 57 is severely defective as an immunomodulator, and the holotoxin exhibits ablated toxicity, and retains the ability to bind with high affinity to GM-1. The invention further discloses that **EtxB** or an **EtxB**(H57A) are able to act as trafficking mols. that facilitates delivery of exogenous epitopes into the endogenous pathway of class I antigen processing and presentation.

L4 ANSWER 3 OF 6 MEDLINE on STN DUPLICATE 2
2003120615. PubMed ID: 12634387. The B subunit of Escherichia coli heat-labile enterotoxin enhances CD8+ cytotoxic-T-lymphocyte killing of Epstein-Barr virus-infected cell lines. Ong Kong-Wee; Wilson A Douglas; Hirst Timothy R; Morgan Andrew J. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, United Kingdom.) Journal of virology, (2003 Apr) 77 (7) 4298-305. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Epstein-Barr virus (EBV) is associated with a number of important human cancers, including nasopharyngeal carcinoma, gastric carcinoma, and Hodgkin's lymphoma. These tumors express a viral nuclear antigen, EBV nuclear antigen 1 (EBNA1), which cannot be presented to T cells in a major histocompatibility complex class I context, and the viral latent membrane proteins (LMPs). Although the LMPs are expressed in these tumors, no effective immune response is made. We report here that exposure to the cholera-like enterotoxin B subunit (**EtxB**) in EBV-infected lymphoblastoid cell lines (LCLs) enhances their susceptibility to killing by LMP-specific CD8(+) cytotoxic T lymphocytes (CTLs) in a HLA class I-restricted manner. CTL killing of LCLs is dramatically increased through both transporter-associated protein-dependent and -independent epitopes after **EtxB treatment**. The use of mutant B subunits revealed that the enhanced susceptibility of LCLs to CTL killing is dependent on the B subunit's interaction with GM(1) but not its signaling properties. These important findings could underpin the

development of novel approaches to treating EBV-associated malignancies and may offer a general approach to increasing the presentation of other tumor and viral antigens.

L4 ANSWER 4 OF 6 MEDLINE on STN DUPLICATE 3
2002150343. PubMed ID: 11882700. Contribution of the ADP-ribosylating and receptor-binding properties of cholera-like enterotoxins in modulating cytokine secretion by human intestinal epithelial cells. Soriani Marco; Bailey Lorna; Hirst Timothy R. (Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD, UK.) Microbiology (Reading, England), (2002 Mar) 148 (Pt 3) 667-76. Journal code: 9430468. ISSN: 1350-0872. Pub. country: England: United Kingdom. Language: English.
AB When epithelial cells first encounter cholera toxin (Ctx) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a number of cytokines that may contribute to the toxin's potent immunomodulatory properties. Much less is known about the ability of the heat-labile enterotoxin of Escherichia coli (Etx), a close homologue of Ctx, to elicit cytokine secretion by epithelial cells. This study shows that **treatment** of human intestinal epithelial T84 cells with Etx induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1alpha and IL-1beta and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the toxin A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive mutant of Etx nor **EtxB** was able to induce cytokine secretion. The behaviour of Ctx and CtxB was very similar to that of Etx and **EtxB**, respectively. The spectrum of cytokines released by Etx and Ctx indicates that the toxins may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.

L4 ANSWER 5 OF 6 MEDLINE on STN DUPLICATE 4
2001392800. PubMed ID: 11447169. Escherichia coli enterotoxin B subunit triggers apoptosis of CD8(+) T cells by activating transcription factor c-myc. Soriani M; Williams N A; Hirst T R. (Department of Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD, United Kingdom.) Infection and immunity, (2001 Aug) 69 (8) 4923-30. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB Heat-labile enterotoxin from enterotoxinogenic Escherichia coli is not only an important cause of diarrhea in humans and domestic animals but also possesses potent immunomodulatory properties. Recently, the nontoxic, receptor-binding B subunit of heat-labile enterotoxin (**EtxB**) was found to induce the selective death of CD8(+) T cells, suggesting that **EtxB** may trigger activation of proapoptotic signaling pathways. Here we show that **EtxB treatment** of CD8(+) T cells but not of CD4(+) T cells triggers the specific up-regulation of the transcription factor c-myc, implicated in the control of cell proliferation, differentiation, and death. A concomitant elevation in Myc protein levels was also evident, with peak expression occurring 4 h posttreatment. Preincubation with c-myc antisense oligodeoxynucleotides demonstrated that Myc expression was necessary for **EtxB**-mediated apoptosis. Myc activation was also associated with an increase of IkappaBalpha turnover, suggesting that elevated Myc expression may be dependent on NF-kappaB. When CD8(+) T cells were pretreated with inhibitors of IkappaBalpha turnover and NF-kappaB translocation, this resulted in a marked reduction in both **EtxB** -induced apoptosis and Myc expression. Further, a non-receptor-binding mutant of **EtxB**, **EtxB**(G33D), was shown to lack the capacity to activate Myc transcription. These findings provide further evidence that **EtxB** is a signaling molecule that triggers activation of transcription factors involved in cell survival.

L4 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 5
2001210820. PubMed ID: 11298654. Cholera toxin and Escherichia coli

enterotoxin B-subunits inhibit macrophage-mediated antigen processing and presentation: evidence for antigen persistence in non-acidic recycling endosomal compartments. Millar D G; Hirst T R. (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, UK.) Cellular microbiology, (2001 May) 3 (5) 311-29. Journal code: 100883691. ISSN: 1462-5814. Pub. country: England: United Kingdom. Language: English.

AB Cholera toxin (Ctx) and the closely related Escherichia coli heat-labile enterotoxin (Etx) not only act as mediators of diarrhoeal disease but also exert potent immunomodulatory properties on mammalian immune systems. The toxins normally exert their diarrhoeagenic effects by initiating receptor-mediated uptake into vesicles that enter a retrograde trafficking pathway, circumventing degradative compartments and targeting them to the trans-Golgi network (TGN) and endoplasmic reticulum. Here, we examine whether receptor-mediated binding and cellular entry by the toxin B-subunits also lead to concomitant changes in uptake and trafficking of exogenous antigens that could contribute to the potent immunomodulatory properties of these toxins. **Treatment** of the macrophage (J774.2) cell line with Etx B-subunit (**EtxB**) resulted in **EtxB** transport to the TGN and also led to the formation of large, translucent, non-acidic, **EtxB**-devoid vacuoles. When exogenous antigens were added, **EtxB**-treated cells were found to be proficient in both internalization of ovalbumin (OVA) and phagocytosis of bacterial particles. However, the internalized OVA, instead of trafficking along a lysosome-directed endocytic pathway via acidified endosomes, persisted in a non-acidic, light-density compartment that was distinct from the translucent vacuoles. The rerouted OVA did not co-localize with the endosomal markers rab5 or rab11, nor with **EtxB**, but was retained in a transferrin receptor-positive compartment. The failure of OVA to enter the late endosomal/lysosomal compartments correlated with a striking inhibition of OVA peptide processing and presentation to OVA-responsive CD4+ T-cells. CtxB also modulated OVA trafficking and inhibited antigen presentation. These findings demonstrate that the B-subunits of Ctx and Etx alter the progression of exogenous antigens along the endocytic processing pathway, and prevent or delay efficient epitope presentation and T-cell stimulation. The formation of such 'antigen depots' could contribute to the immunomodulatory properties of these bacterial virulence determinants.

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L5            0 L1 AND ALLERGEN

=> s l1 and type I allergies
L6            0 L1 AND TYPE I ALLERGIES

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L7         9247 (WILLIAMS N?/AU OR HIRST T?/AU OR BIENENSTOCK J?/AU)

=> s l7 and EtxB
L8          217 L7 AND ETXB

=> dup remove l8
PROCESSING COMPLETED FOR L8
L9            62 DUP REMOVE L8 (155 DUPLICATES REMOVED)

=> d l9 1-62 cbib abs

L9   ANSWER 1 OF 62     MEDLINE on STN                    DUPLICATE 1
2004590487.     PubMed ID: 15342647.   Trafficking of Exogenous Peptides into
     Proteasome-dependent Major Histocompatibility Complex Class I Pathway
     following Enterotoxin B Subunit-mediated Delivery. Hearn Arron R; de Haan
     Lolke; Pemberton Alexander J; Hirst Timothy R; Rivett A
     Jennifer. (Departments of Biochemistry and Pathology and Microbiology,
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School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, United Kingdom.) Journal of biological chemistry, (2004 Dec 3) 279 (49) 51315-22. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The B-subunit component of Escherichia coli heat-labile enterotoxin (**EtxB**), which binds to cell surface GM1 ganglioside receptors, was recently shown to be a highly effective vehicle for delivery of conjugated peptides into the major histocompatibility complex (MHC) class I pathway. In this study we have investigated the pathway of epitope delivery. The peptides used contained the epitope either located at the C terminus or with a C-terminal extension. Pretreatment of cells with cholesterol-disrupting agents blocked transport of **EtxB** conjugates to the Golgi/endoplasmic reticulum, but did not affect **EtxB**-mediated MHC class I presentation. Under these conditions, **EtxB** conjugates entered EEA1-positive early endosomes where peptides were cleaved and translocated into the cytosol. Endosome acidification was required for epitope presentation. Purified 20 S immunoproteasomes were able to generate the epitope from peptides in vitro, but 26 S proteasomes were not. Only presentation from the C-terminal extended peptide was proteasome-dependent in cells, and this was found to be significantly slower than presentation from peptides with the epitope at the C terminus. These results implicate the proteasome in the generation of the correct C terminus of the epitope and are consistent with proteasome-independent N-terminal trimming. Epitope presentation was blocked in a TAP-deficient cell line, providing further evidence that conjugated peptides enter the cytosol as well as demonstrating a requirement for the peptide transporter. Our findings demonstrate the utility of **EtxB**-mediated peptide delivery for rapid and efficient loading of MHC class I epitopes in several different cell types. Conjugated peptides are released from early endosomes into the cytosol where they gain access to proteasomes and TAP in the "classical" pathway of class I presentation.

L9 ANSWER 2 OF 62 MEDLINE on STN DUPLICATE 2

2004474715. PubMed ID: 15385486. The B subunit of Escherichia coli heat-labile enterotoxin induces both caspase-dependent and -independent cell death pathways in CD8+ T cells. Salmond Robert J; Williams Rachel; **Hirst Timothy R**; **Williams Neil A**. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, United Kingdom.) Infection and immunity, (2004 Oct) 72 (10) 5850-7. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The nontoxic B subunit of Escherichia coli heat-labile enterotoxin (**EtxB**) is a potent immunomodulatory molecule that acts both as an adjuvant and to stimulate immune deviation processes, resulting in the suppression of Th1-associated inflammatory responses. The ability of **EtxB** to alter immune reactivity is dependent on its ability to modulate immune cell function through binding to cell surface molecules, the principal receptor of which is the ubiquitous GM1-ganglioside. **EtxB** activates B cells and antigen-presenting cells and induces the selective apoptosis of murine CD8+ T cells. We postulated that these effects are mediated by the induction of intracellular signaling pathways following **EtxB**-receptor interaction. We have previously shown that CD8+ T-cell apoptosis induced by **EtxB** results from the activation of the transcription factor NF-kappaB and caspases. Here we report that while caspase activity is required for apoptosis, additional features of cell death are caspase independent. **EtxB** induces a rapid loss of mitochondrial membrane potential and cell viability that are unaffected by caspase inhibitors. In addition, our data suggest that these processes are independent of the activity of Bax and Bcl-2 but are mediated by nitric oxide synthase.

L9 ANSWER 3 OF 62 MEDLINE on STN DUPLICATE 3

2004311350. PubMed ID: 15213152. Nasal delivery of antigen with the B

subunit of Escherichia coli heat-labile enterotoxin augments antigen-specific T-cell clonal expansion and differentiation. Apostolaki Maria; **Williams Neil A.** (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol, United Kingdom.) Infection and immunity, (2004 Jul) 72 (7) 4072-80. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Escherichia coli heat-labile enterotoxin has unique immunogenic and adjuvant properties when administered mucosally to mice. These properties have revealed the potential for its use in the development of mucosal vaccines, an area of increasing interest. However, the inherent toxicity mediated by the A subunit precludes its widespread use. This problem has led to attempts to dissociate toxicity from adjuvant function by use of the B subunit. The ability of the B subunit of E. coli heat-labile enterotoxin (**EtxB**) to enhance responses against antigens coadministered intranasally is demonstrated here with the use of the DO11.10 adoptive-transfer model, in which ovalbumin (OVA)-specific adoptively transferred T cells can be monitored directly by flow cytometry. Intranasal delivery of OVA with **EtxB** resulted in increased T-cell proliferative and systemic antibody responses against antigens. The increased Th2 cytokine production detected following in vitro restimulation of splenocyte and cervical lymph node (CLN) cells from the immunized mice correlated with increased OVA-specific immunoglobulin G1 antibody production. Flow cytometric analysis of T cells from mice early after immunization directly revealed the ability of **EtxB** to support antigen-specific clonal expansion and differentiation. Furthermore, while responses were first detected in the CLNs, they rapidly progressed to the spleen, where they were further sustained. Examination of CD69 expression on dividing cells supported the notion that activation induced by the presence of antigens is not sufficient to drive T-cell differentiation. Furthermore, a lack of CD25 expression on dividing cells suggested that **EtxB**-mediated T-cell clonal expansion may occur without a sustained requirement for interleukin 2.

L9 ANSWER 4 OF 62 MEDLINE on STN DUPLICATE 4

2004457371. PubMed ID: 15364452. Intranasal immunisation of mice with liposomes containing recombinant meningococcal OpaB and OpaJ proteins. de Jonge Marien I; Hamstra Hendrik Jan; Jiskoot Wim; Roholl Paul; **Williams Neil A**; Dankert Jacob; van Alphen Loek; van der Ley Peter. (Netherlands Vaccine Institute (NVI), 3720 AL Bilthoven, The Netherlands.) Vaccine, (2004 Sep 28) 22 (29-30) 4021-8. Journal code: 8406899. ISSN: 0264-410X. Pub. country: Netherlands. Language: English.

AB The opacity (Opa) proteins of Neisseria meningitidis are outer membrane proteins involved in adhesion and invasion of host epithelial cells and are therefore expected to play an important role in colonisation of the nasopharynx. The majority of meningococcal Opa proteins bind to members of the CEACAM receptor family, such as CEA. Blocking of the Opa-CEACAM interaction by mucosal anti-Opa antibodies could thus constitute an important protective mechanism for novel meningococcal vaccines. In this study we analysed the specific anti-Opa antibody responses after intranasal immunisation of mice with liposomes containing purified and native OpaB (recognising the CEA receptor) and OpaJ (no affinity for CEA) proteins. These antigens were combined with or without one of three different adjuvants, i.e. purified meningococcal LPS, monophosphoryl lipid A (MPL) or the B-subunit of Escherichia coli heat-labile enterotoxin (**EtxB**). After intranasal immunisation with any of these formulations, anti-Opa IgA antibodies were found in nasal lavages and in some cases anti-Opa IgA and IgG antibodies were also found in lung lavages. With OpaJ but not OpaB, significant bactericidal serum titres were obtained. Of the different adjuvants used, meningococcal LPS gave the strongest overall immune response. Non-adjuvated liposomal Opa formulations were poorly immunogenic. No differences were found between the immune response in transgenic mice expressing the CEA-receptor and non-transgenic mice, showing that the CEA-Opa interaction does not

influence the antibody response.

L9 ANSWER 5 OF 62 MEDLINE on STN DUPLICATE 5
2004257063. PubMed ID: 15155624. Recombinant Streptococcus equi proteins protect mice in challenge experiments and induce immune response in horses. Flock Margareta; Jacobsson Karin; Frykberg Lars; **Hirst Timothy R**; Franklin Anders; Guss Bengt; Flock Jan-Ingmar. (Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden.) Infection and immunity, (2004 Jun) 72 (6) 3228-36. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Horses that have undergone infection caused by Streptococcus equi subspecies equi (strangles) were found to have significantly increased serum antibody titers against three previously characterized proteins, FNZ (cell surface-bound fibronectin binding protein), SFS (secreted fibronectin binding protein), and EAG (alpha2-macroglobulin, albumin, and immunoglobulin G [IgG] binding protein) from S. equi. To assess the protective efficacy of vaccination with these three proteins, a mouse model of equine strangles was utilized. Parts of the three recombinant proteins were used to immunize mice, either subcutaneously or intranasally, prior to nasal challenge with S. equi subsp. equi. The adjuvant used was **EtxB**, a recombinant form of the B subunit of Escherichia coli heat-labile enterotoxin. It was shown that nasal colonization of S. equi subsp. equi and weight loss due to infection were significantly reduced after vaccination compared with a mock-vaccinated control group. This effect was more pronounced after intranasal vaccination than after subcutaneous vaccination; nearly complete eradication of nasal colonization was obtained after intranasal vaccination ($P < 0.001$). When the same antigens were administered both intranasally and subcutaneously to healthy horses, significant mucosal IgA and serum IgG antibody responses against FNZ and EAG were obtained. The antibody response was enhanced when **EtxB** was used as an adjuvant. No adverse effects of the antigens or **EtxB** were observed. Thus, FNZ and EAG in conjunction with **EtxB** are promising candidates for an efficacious and safe vaccine against strangles.

L9 ANSWER 6 OF 62 MEDLINE on STN DUPLICATE 6
2004077030. PubMed ID: 14965302. Modulation of the immune response by the cholera-like enterotoxins. Plant Andrea; **Williams Neil A**. (University of Bristol, Department of Pathology and Microbiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK.) Current topics in medicinal chemistry, (2004) 4 (5) 509-19. Ref: 112. Journal code: 101119673. ISSN: 1568-0266. Pub. country: Netherlands. Language: English.

AB Cholera toxins and heat labile enterotoxin from E. coli differ from most soluble proteins in eliciting systemic immunity both against themselves and unrelated admixed antigens, rather than tolerance following administration to a mucosal surface. Several reports have also demonstrated preferential induction of Th2-type responses when these molecules are used as adjuvants. Conversely, these proteins and their non-toxic derivatives, including the B sub-units are also able prevent and alleviate autoimmune diseases in naive and systemically immune hosts demonstrating wide-ranging effects on the immune system. The recent observation that amelioration of autoimmune disease is associated with the generation of regulatory T cells which inhibit pathogenic Th1 responses may also help to consolidate these two apparently contradictory outcomes of exposure to the cholera-like enterotoxins. Furthermore, the observation that **EtxB** is able to alleviate autoimmune disease in the absence of conjugation to autoantigen highlights its potential for use in the clinical setting where the target antigen is often unknown. Direct effects on T cells, B cells and APC have been demonstrated in vitro which have provided insights into how these molecules may elicit these diverse effects. Further investigation is required for elucidation of the mechanisms of action of adjuvanticity and tolerance induction by these

molecules to realise their potential for use in vaccines and therapies for autoimmune disease in humans.

L9 ANSWER 7 OF 62 CAPLUS COPYRIGHT 2004 ACS on STN
2003:6139 Document No. 138:68275 Mutant forms of enterotoxin (**EtxB**) and cholera toxin (CtxB), and their therapeutic uses as target site-specific carriers. **Hirst, Timothy Raymond** (University of Bristol, UK). PCT Int. Appl. WO 2003000899 A1 20030103, 84 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-GB2829 20020620. PRIORITY: GB 2001-15382 20010622.
AB The present invention describes the use of a mutant form of enterotoxin subunit B (**EtxB**) or cholera toxin subunit B (CtxB) to deliver an agent to a target cell wherein the mutant has GM-1 binding activity, and a reduced immunogenic and immunomodulatory activity relative to the wild type form of **EtxB** or CtxB. Specifically, the mutant CtxB with His to Ala substitution at position 57 is severely defective as an immunomodulator, and the holotoxin exhibits ablated toxicity, and retains the ability to bind with high affinity to GM-1. The invention further discloses that **EtxB** or an **EtxB**(H57A) are able to act as trafficking mols. that facilitates delivery of exogenous epitopes into the endogenous pathway of class I antigen processing and presentation.

L9 ANSWER 8 OF 62 MEDLINE on STN DUPLICATE 7
2003120615. PubMed ID: 12634387. The B subunit of Escherichia coli heat-labile enterotoxin enhances CD8+ cytotoxic-T-lymphocyte killing of Epstein-Barr virus-infected cell lines. Ong Kong-Wee; Wilson A Douglas; **Hirst Timothy R**; Morgan Andrew J. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, United Kingdom.) Journal of virology, (2003 Apr) 77 (7) 4298-305. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Epstein-Barr virus (EBV) is associated with a number of important human cancers, including nasopharyngeal carcinoma, gastric carcinoma, and Hodgkin's lymphoma. These tumors express a viral nuclear antigen, EBV nuclear antigen 1 (EBNA1), which cannot be presented to T cells in a major histocompatibility complex class I context, and the viral latent membrane proteins (LMPs). Although the LMPs are expressed in these tumors, no effective immune response is made. We report here that exposure to the cholera-like enterotoxin B subunit (**EtxB**) in EBV-infected lymphoblastoid cell lines (LCLs) enhances their susceptibility to killing by LMP-specific CD8(+) cytotoxic T lymphocytes (CTLs) in a HLA class I-restricted manner. CTL killing of LCLs is dramatically increased through both transporter-associated protein-dependent and -independent epitopes after **EtxB** treatment. The use of mutant B subunits revealed that the enhanced susceptibility of LCLs to CTL killing is dependent on the B subunit's interaction with GM(1) but not its signaling properties. These important findings could underpin the development of novel approaches to treating EBV-associated malignancies and may offer a general approach to increasing the presentation of other tumor and viral antigens.

L9 ANSWER 9 OF 62 MEDLINE on STN DUPLICATE 8
2003501116. PubMed ID: 14579287. The B subunit of Escherichia coli heat labile enterotoxin abrogates oral tolerance, promoting predominantly Th2-type immune responses. Plant Andrea; Williams Rachel; Jackson Michelle E; **Williams Neil A**. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, GB.) European journal of immunology, (2003 Nov) 33 (11) 3186-95. Journal code: 1273201.

ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Mucosal antigen encounter usually results in a state of systemic non-responsiveness (tolerance). This failure to mount a protective response is a major hurdle to mucosal vaccine development. Hence, the identification of safe and effective mucosal adjuvants promoting protective immunity is of critical importance. The non-toxic B subunit of Escherichia coli heat labile enterotoxin(**EtxB**) is a potent nasal adjuvant; however, its usefulness following oral delivery is unconfirmed. We used DO11.10 chimeric mice to assess whether **EtxB** could abrogate tolerance to oral OVA. We show that admixing **EtxB** with OVA for oral immunization abrogates oral tolerance and results in a weak anti-OVA immune response. Importantly, **EtxB** profoundly modulated the nature of the response to subsequent parenteral challenge, promoting IgG1 in favor of IgG2a antibodies and depressing IFN-gamma production while elevating TGF-beta secretion. The addition of **EtxB** promoted T cell division, as assessed by loss of staining with carboxyfluorescein diacetate succinimidyl ester. Enhanced cell division promoted by **EtxB** was associated with T cell differentiation (increased numbers of CD45RBlow cells) in vivo, although dividing OVA-specific T cells were CD25-. These data suggest that although **EtxB** is a weak oral adjuvant, it can profoundly modulate the nature of the immune response to admixed antigen.

L9 ANSWER 10 OF 62 MEDLINE on STN DUPLICATE 9

2003084109. PubMed ID: 12595472. Mutant Escherichia coli heat-labile toxin B subunit that separates toxoid-mediated signaling and immunomodulatory action from trafficking and delivery functions. Fraser Sylvia A; de Haan Lolke; Hearn Arron R; Bone Heather K; Salmond Robert J; Rivett A Jennifer; **Williams Neil A**; **Hirst Timothy R**. (Department of Pathology & Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, United Kingdom.) Infection and immunity, (2003 Mar) 71 (3) 1527-37. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The homopentameric B-subunit components of Escherichia coli heat-labile enterotoxin (**EtxB**) and cholera toxin (CtxB) possess the capacity to enter mammalian cells and to activate cell-signaling events in leukocytes that modulate immune cell function. Both properties have been attributed to the ability of the B subunits to bind to GM1-ganglioside receptors, a ubiquitous glycosphingolipid found in the plasma membrane. Here we describe the properties of **EtxB**(H57S), a mutant B subunit with a His-->Ser substitution at position 57. The mutant was found to be severely defective in inducing leukocyte signaling, as shown by failure to (i) trigger caspase 3-mediated CD8(+)-T-cell apoptosis, (ii) activate nuclear translocation of NF-kappaB in Jurkat T cells, (iii) induce a potent anti-B-subunit response in mice, or (iv) serve as a mucosal adjuvant. However, its GM1 binding, cellular uptake, and delivery functions remained intact. This was further validated by the finding that **EtxB**(H57S) was as effective as **EtxB** in delivering a conjugated model class I epitope into the major histocompatibility complex class I pathway of a dendritic cell line. These observations imply that GM1 binding alone is not sufficient to trigger the signaling events responsible for the potent immunomodulatory properties of **EtxB**. Moreover, they demonstrate that its signaling properties play no role in **EtxB** uptake and trafficking. Thus, **EtxB**(H57S) represents a novel tool for evaluating the complex cellular interactions and signaling events occurring after receptor interaction, as well as offering an alternative means of delivering attached peptides in the absence of the potent immunomodulatory signals induced by wild-type B subunits.

L9 ANSWER 11 OF 62 MEDLINE on STN DUPLICATE 10

2003323855. PubMed ID: 12853160. Selective induction of CD8+CD4- thymocyte apoptosis mediated by the B-subunit of Escherichia coli heat-labile

enterotoxin. Salmond Robert J; Williams Rachel; **Hirst Timothy R**; **Williams Neil A**. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD, UK.) Immunology letters, (2003 Jul 3) 88 (1) 43-6. Journal code: 7910006. ISSN: 0165-2478. Pub. country: Netherlands. Language: English.

AB Receptor-binding by the B-subunit of Escherichia coli heat-labile enterotoxin (**EtxB**) induces apoptosis of peripheral CD8(+), but not CD4(+) T-cells. Given that peripheral CD8(+) and CD4(+) T cells arise from a common developmental pathway in the thymus, we investigated the effects of **EtxB** on different thymocyte populations. We show that the acquisition of sensitivity to **EtxB**-mediated cell death arises following transition of CD4(+)CD8(+) double positive cells into the CD4(-)CD8(+) pathway. Maturation of T cells into CD4(-)CD8(+) single positive cells is associated with upregulated expression of receptors for **EtxB**.

L9 ANSWER 12 OF 62 MEDLINE on STN DUPLICATE 11

2002271835. PubMed ID: 12011020. Enhanced delivery of exogenous peptides into the class I antigen processing and presentation pathway. De Haan Lolke; Hearn Arron R; Rivett A Jennifer; **Hirst Timothy R**. (Department of Pathology & Microbiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom.) Infection and immunity, (2002 Jun) 70 (6) 3249-58. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Current immunization strategies, using peptide or protein antigens, generally fail to elicit cytotoxic-T-lymphocyte responses, since these antigens are unable to access intracellular compartments where loading of major histocompatibility complex class I (MHC-I) molecules occurs. In an attempt to circumvent this, we investigated whether the GM1 receptor-binding B subunit of Escherichia coli heat-labile toxin (**EtxB**) could be used to deliver class I epitopes. When a class I epitope was conjugated to **EtxB**, it was delivered into the MHC-I presentation pathway in a GM1-binding-dependent fashion and resulted in the appearance of MHC-I-epitope complexes at the cell surface. Importantly, we show that the efficiency of **EtxB**-mediated epitope delivery could be strikingly enhanced by incorporating, adjacent to the class I epitope, a 10-amino-acid segment from the C terminus of the DNA polymerase (Pol) of herpes simplex virus. The replacement of this 10-amino-acid segment by a heterologous sequence or the introduction of specific amino acid substitutions within this segment either abolished or markedly reduced the efficiency of class I epitope delivery. If the epitope was extended at its C terminus, **EtxB**-mediated delivery into the class I presentation pathway was found to be completely dependent on proteasome activity. Thus, by combining the GM1-targeting function of **EtxB** with the 10-amino-acid Pol segment, highly efficient delivery of exogenous epitopes into the endogenous pathway of class I antigen processing and presentation can be achieved.

L9 ANSWER 13 OF 62 MEDLINE on STN DUPLICATE 12

2002368102. PubMed ID: 12115657. CD8+ T cell apoptosis induced by Escherichia coli heat-labile enterotoxin B subunit occurs via a novel pathway involving NF-kappaB-dependent caspase activation. Salmond Robert J; Pitman Richard S; Jimi Eijiro; Soriani Marco; **Hirst Timothy R** ; Ghosh Sankar; Rincon Mercedes; **Williams Neil A**. (University of Bristol, Department of Pathology and Microbiology, School of Medical Sciences, Bristol, GB.) European journal of immunology, (2002 Jun) 32 (6) 1737-47. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB The B subunit of Escherichia coli heat-labile enterotoxin (**EtxB**) is a potent immunomodulatory molecule capable of treating and preventing autoimmune disease. These properties result from its ability to bind to glycolipid receptors, principally G(M1) ganglioside, and modulate immune cell function. **EtxB** receptor binding causes B cell activation, modulates monocyte cytokine secretion and triggers apoptosis of CD8+ T

cells. These wide-ranging effects suggest that B subunit receptor interaction triggers signaling events affecting cellular differentiation. We have investigated the processes by which **EtxB** induces CD8+ T cell apoptosis. We show that receptor interaction by **EtxB** activates caspase-3 in CD8+ but not in CD4+ T cells. Inhibition of caspase-3 blocks the apoptotic process. **EtxB** induces the activation of NF-kappaB in both CD8+ and CD4+ T cells. The findings that (i) SN50, a peptide inhibitor of NF-kappaB nuclear translocation, prevents caspase-3 activation and subsequent apoptosis, and (ii) CD8+CD4- thymocytes from transgenic mice expressing a dominant-negative form of the IkappaBalpha protein were markedly less susceptible to **EtxB** -induced apoptosis than cells from wild-type mice, indicate that NF-kappaB is important in the induction of the apoptotic pathway. Further investigations revealed that while caspase-8 activity is detected concomitant to caspase-3, caspase-9 activation, following mitochondrial cytochrome c release, is detectable later on. These observations are consistent with death receptor-mediated signaling, however, experiments using lpr/lpr and p55 TNFR -/- mice rule out the involvement of Fas and the p55 TNF receptor, respectively. The data therefore indicate that **EtxB**-mediated apoptosis occurs via a novel pathway involving NF-kappaB.

L9 ANSWER 14 OF 62 MEDLINE on STN DUPLICATE 13

2002372838. PubMed ID: 12115200. Escherichia coli heat-labile enterotoxin B subunit prevents autoimmune arthritis through induction of regulatory CD4+ T cells. Luross Jeffrey A; Heaton Tricia; **Hirst Timothy R**; Day Michael J; **Williams Neil A**. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK.) Arthritis and rheumatism, (2002 Jun) 46 (6) 1671-82. Journal code: 0370605. ISSN: 0004-3591. Pub. country: United States. Language: English.

AB OBJECTIVE: The receptor-binding B subunit of Escherichia coli heat-labile enterotoxin (**EtxB**) is a highly stable, nontoxic protein that is capable of modulating immune responses. This study was conducted to determine whether mucosal administration of **EtxB** can block collagen-induced arthritis (CIA) and to investigate the mechanisms involved. METHODS: Clinical arthritis in DBA/1 mice was monitored following mucosal administration of **EtxB** on 4 occasions. The dependence of disease prevention on receptor binding by **EtxB** and the associated alterations to the immune response to type II collagen (CII) were assessed. Adoptive transfer experiments and lymph node cell cocultures were used to investigate the underlying mechanisms. RESULTS: Both intranasal and intragastric delivery of **EtxB** were effective in preventing CIA; a 1-microg dose of **EtxB** was protective after intranasal administration. A non-receptor-binding mutant of **EtxB** failed to prevent disease. Intranasal **EtxB** lowered both the incidence and severity of arthritis when given either at the time of disease induction or 25 days later. **EtxB** markedly reduced levels of anti-CII IgG2a antibodies and interferon-gamma (IFNgamma) production while not affecting levels of IgG1, interleukin-4 (IL-4), or IL-10. Disease protection could be transferred by CD4+ T cells from treated mice, an effect that was abrogated upon depletion of the CD25+ population. In addition, CD4+CD25+ T cells from treated mice were able to suppress anti-CII IFNgamma production by CII-primed lymph node cells. CONCLUSION: Mucosal administration of **EtxB** can be used to prevent or treat CIA. Modulation of the anti-CII immune response by **EtxB** is associated with a reduction in Th1 cell reactivity without a concomitant shift toward Th2. Instead, **EtxB** mediates its effects through enhancing the activity of a population of CD4+ regulatory T cells.

L9 ANSWER 15 OF 62 MEDLINE on STN DUPLICATE 14

2002150343. PubMed ID: 11882700. Contribution of the ADP-ribosylating and receptor-binding properties of cholera-like enterotoxins in modulating

cytokine secretion by human intestinal epithelial cells. Soriani Marco; Bailey Lorna; **Hirst Timothy R**. (Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD, UK.) Microbiology (Reading, England), (2002 Mar) 148 (Pt 3) 667-76. Journal code: 9430468. ISSN: 1350-0872. Pub. country: England: United Kingdom. Language: English.

AB When epithelial cells first encounter cholera toxin (Ctx) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a number of cytokines that may contribute to the toxin's potent immunomodulatory properties. Much less is known about the ability of the heat-labile enterotoxin of Escherichia coli (Etx), a close homologue of Ctx, to elicit cytokine secretion by epithelial cells. This study shows that treatment of human intestinal epithelial T84 cells with Etx induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1alpha and IL-1beta and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the toxin A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive mutant of Etx nor **EtxB** was able to induce cytokine secretion. The behaviour of Ctx and CtxB was very similar to that of Etx and **EtxB**, respectively. The spectrum of cytokines released by Etx and Ctx indicates that the toxins may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.

L9 ANSWER 16 OF 62 MEDLINE on STN DUPLICATE 15

2002298918. PubMed ID: 12039916. Modulation of B lymphocyte signalling by the B subunit of Escherichia coli heat-labile enterotoxin. Bone Heather; Eckholdt Stephanie; **Williams Neil A**. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK.) International immunology, (2002 Jun) 14 (6) 647-58. Journal code: 8916182. ISSN: 0953-8178. Pub. country: England: United Kingdom. Language: English.

AB The non-toxic B subunit of Escherichia coli heat-labile enterotoxin (**EtxB**) is a potent mucosal adjuvant and immunomodulator capable of blocking autoimmune disease. These effects are linked with its ability to modulate lymphocyte populations--a feature that is dependent on binding to ubiquitously expressed cell surface receptors. Here, we demonstrate that **EtxB** can trigger up-regulated expression of class II MHC and CD25 on purified populations of B lymphocytes, suggesting that **EtxB** can directly activate biochemical signalling pathways in these cells. The nature of the intracellular signalling events was investigated. B cells cultured with **EtxB**, but not a non-receptor binding mutant protein, **EtxB**(G33D), caused the activation of the extracellular signal-regulated kinase (Erk) forms of mitogen-activated protein (MAP) kinase in a process that was dependent on MAPK/Erk kinase (MEK), phosphoinositide 3-kinase (PI3-kinase) and protein kinase C (PKC), as determined by the use of specific inhibitors. PI3-kinase was critical not only in the activation of MAP kinase but also in the up-regulation of both class II and CD25. However, MEK inhibition only partially abrogated the **EtxB**-mediated up-regulation of MHC class II expression and did not affect CD25 expression--findings suggesting that additional pathways downstream of PI3-kinase are involved. A role for PKC in these processes was suggested by the finding that inhibitors of PKC completely blocked **EtxB**-mediated CD25 up-regulation. Thus, we have shown that receptor binding by **EtxB** triggers multiple signalling pathways in B cells that regulate the expression of key cell surface molecules.

L9 ANSWER 17 OF 62 MEDLINE on STN DUPLICATE 16

2002357108. PubMed ID: 12100719. Modulation of human monocytes by Escherichia coli heat-labile enterotoxin B-subunit; altered cytokine production and its functional consequences. Turcanu Victor; **Hirst Timothy R**; **Williams Neil A**. (University of Bristol, Department of Pathology and Microbiology, School of Medical Sciences, UK.) Immunology, (2002 Jul) 106 (3) 316-25. Journal code: 0374672. ISSN:

0019-2805. Pub. country: England: United Kingdom. Language: English.

AB In murine systems, the B subunit of Escherichia coli heat-labile enterotoxin (**EtxB**) is a potent immunomodulator capable of suppressing Th1-mediated autoimmune diseases. This results from its ability to bind cell surface receptors, principally GM1-ganglioside, and as a consequence down-regulate the pathological T helper type 1 (Th1) response. The capacity of **EtxB** to alter human T-cell responses has not been investigated. Here we show that **EtxB**, but not the receptor non-binding mutant **EtxB** (G33D), triggers the release of interleukin (IL)-10, IL-6 and tumour necrosis factor-alpha (TNF-alpha) by human monocytes. The production of IL-8, transforming growth factor-beta (TGF-beta) or IL-12 was not enhanced by **EtxB**. Indeed, **EtxB** was shown to inhibit IL-12 secretion in monocytes stimulated with interferon-gamma (IFN-gamma) and lipopolysaccharide (LPS) by an IL-10-independent mechanism. When **EtxB**-treated monocytes were used as antigen presenting cells in an allogeneic mixed lymphocyte reaction (MLR), IL-10 and IFN-gamma production were increased in comparison to levels seen in cultures stimulated with untreated monocytes; proliferation was unaltered. This modulation of the T-cell response was not only evident in the primary MLR triggered by **EtxB**-treated monocytes, but also upon restimulation of the responding T cells with fresh untreated monocytes; indicating that presentation by **EtxB** -treated monocytes leads to altered T-cell differentiation. Sorting experiments showed that IL-10 secreting T cells from the MLR cultures were strong suppressors of T-cell proliferation following their addition into a fresh primary MLR.

L9 ANSWER 18 OF 62 MEDLINE on STN DUPLICATE 17

2001419634. PubMed ID: 11447291. A mutant cholera toxin B subunit that binds GM1- ganglioside but lacks immunomodulatory or toxic activity. Aman A T; Fraser S; Merritt E A; Rodigherio C; Kenny M; Ahn M; Hol W G; **Williams N A**; Lencer W I; **Hirst T R**. (Department of Pathology and Microbiology, University of Bristol, Bristol BS81TD, United Kingdom.) Proceedings of the National Academy of Sciences of the United States of America, (2001 Jul 17) 98 (15) 8536-41. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB GM1-ganglioside receptor binding by the B subunit of cholera toxin (CtxB) is widely accepted to initiate toxin action by triggering uptake and delivery of the toxin A subunit into cells. More recently, GM1 binding by isolated CtxB, or the related B subunit of Escherichia coli heat-labile enterotoxin (**EtxB**), has been found to modulate leukocyte function, resulting in the down-regulation of proinflammatory immune responses that cause autoimmune disorders such as rheumatoid arthritis and diabetes. Here, we demonstrate that GM1 binding, contrary to expectation, is not sufficient to initiate toxin action. We report the engineering and crystallographic structure of a mutant cholera toxin, with a His to Ala substitution in the B subunit at position 57. Whereas the mutant retained pentameric stability and high affinity binding to GM1-ganglioside, it had lost its immunomodulatory activity and, when part of the holotoxin complex, exhibited ablated toxicity. The implications of these findings on the mode of action of cholera toxin are discussed.

L9 ANSWER 19 OF 62 MEDLINE on STN DUPLICATE 18

2001392800. PubMed ID: 11447169. Escherichia coli enterotoxin B subunit triggers apoptosis of CD8(+) T cells by activating transcription factor c-myc. Soriani M; **Williams N A**; **Hirst T R**. (Department of Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD, United Kingdom.) Infection and immunity, (2001 Aug) 69 (8) 4923-30. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Heat-labile enterotoxin from enterotoxinogenic Escherichia coli is not only an important cause of diarrhea in humans and domestic animals but also possesses potent immunomodulatory properties. Recently, the nontoxic, receptor-binding B subunit of heat-labile enterotoxin (

EtxB) was found to induce the selective death of CD8(+) T cells, suggesting that **EtxB** may trigger activation of proapoptotic signaling pathways. Here we show that **EtxB** treatment of CD8(+) T cells but not of CD4(+) T cells triggers the specific up-regulation of the transcription factor c-myc, implicated in the control of cell proliferation, differentiation, and death. A concomitant elevation in Myc protein levels was also evident, with peak expression occurring 4 h posttreatment. Preincubation with c-myc antisense oligodeoxynucleotides demonstrated that Myc expression was necessary for **EtxB**-mediated apoptosis. Myc activation was also associated with an increase of IkappaBalpha turnover, suggesting that elevated Myc expression may be dependent on NF-kappaB. When CD8(+) T cells were pretreated with inhibitors of IkappaBalpha turnover and NF-kappaB translocation, this resulted in a marked reduction in both **EtxB**-induced apoptosis and Myc expression. Further, a non-receptor-binding mutant of **EtxB**, **EtxB**(G33D), was shown to lack the capacity to activate Myc transcription. These findings provide further evidence that **EtxB** is a signaling molecule that triggers activation of transcription factors involved in cell survival.

L9 ANSWER 20 OF 62 MEDLINE on STN DUPLICATE 19

2001248162. PubMed ID: 11292779. Escherichia coli heat-labile enterotoxin B subunit is a more potent mucosal adjuvant than its vlosely related homologue, the B subunit of cholera toxin. Millar D G; **Hirst T R** ; Snider D P. (Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.. dmillar@uhnres.utoronto.ca) . Infection and immunity, (2001 May) 69 (5) 3476-82. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Although cholera toxin (Ctx) and Escherichia coli heat-labile enterotoxin (Etx) are known to be potent mucosal adjuvants, it remains controversial whether the adjuvanticity of the holotoxins extends to their nontoxic, receptor-binding B subunits. Here, we have systematically evaluated the comparative adjuvant properties of highly purified recombinant **EtxB** and CtxB. **EtxB** was found to be a more potent adjuvant than CtxB, stimulating responses to hen egg lysozyme when the two were coadministered to mice intranasally, as assessed by enhanced serum and secretory antibody titers as well as by stimulation of lymphocyte proliferation in spleen and draining lymph nodes. These results indicate that, although structurally very similar, **EtxB** and CtxB have strikingly different immunostimulatory properties and should not be considered equivalent as prospective vaccine adjuvants.

L9 ANSWER 21 OF 62 MEDLINE on STN DUPLICATE 20

2001210820. PubMed ID: 11298654. Cholera toxin and Escherichia coli enterotoxin B-subunits inhibit macrophage-mediated antigen processing and presentation: evidence for antigen persistence in non-acidic recycling endosomal compartments. Millar D G; **Hirst T R**. (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, UK.) Cellular microbiology, (2001 May) 3 (5) 311-29. Journal code: 100883691. ISSN: 1462-5814. Pub. country: England: United Kingdom. Language: English.

AB Cholera toxin (Ctx) and the closely related Escherichia coli heat-labile enterotoxin (Etx) not only act as mediators of diarrhoeal disease but also exert potent immunomodulatory properties on mammalian immune systems. The toxins normally exert their diarrhoeagenic effects by initiating receptor-mediated uptake into vesicles that enter a retrograde trafficking pathway, circumventing degradative compartments and targeting them to the trans-Golgi network (TGN) and endoplasmic reticulum. Here, we examine whether receptor-mediated binding and cellular entry by the toxin B-subunits also lead to concomitant changes in uptake and trafficking of exogenous antigens that could contribute to the potent immunomodulatory properties of these toxins. Treatment of the macrophage (J774.2) cell line with Etx B-subunit (**EtxB**) resulted in **EtxB**

transport to the TGN and also led to the formation of large, translucent, non-acidic, **EtxB**-devoid vacuoles. When exogenous antigens were added, **EtxB**-treated cells were found to be proficient in both internalization of ovalbumin (OVA) and phagocytosis of bacterial particles. However, the internalized OVA, instead of trafficking along a lysosome-directed endocytic pathway via acidified endosomes, persisted in a non-acidic, light-density compartment that was distinct from the translucent vacuoles. The rerouted OVA did not co-localize with the endosomal markers rab5 or rab11, nor with **EtxB**, but was retained in a transferrin receptor-positive compartment. The failure of OVA to enter the late endosomal/lysosomal compartments correlated with a striking inhibition of OVA peptide processing and presentation to OVA-responsive CD4+ T-cells. CtxB also modulated OVA trafficking and inhibited antigen presentation. These findings demonstrate that the B-subunits of Ctx and Etx alter the progression of exogenous antigens along the endocytic processing pathway, and prevent or delay efficient epitope presentation and T-cell stimulation. The formation of such 'antigen depots' could contribute to the immunomodulatory properties of these bacterial virulence determinants.

L9 ANSWER 22 OF 62 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2001:220859 Document No.: PREV200100220859. Immunomodulation of the human MLR by E. coli - heat-labile toxin B subunit (**EtxB**): Induction of regulatory T cells. **Williams, Neil A.** [Reprint author]; Turcanu, Victor [Reprint author]. Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 ITD, UK. Diabetes-Metabolism Research and Reviews, (January-February, 2001) Vol. 17, No. Suppl. 1, pp. S37. print.
Meeting Info.: 5th International Congress of the Immunology of Diabetes Society. Madras, Chennai, India. February 13-16, 2001. Immunology of Diabetes Society.
ISSN: 1520-7552. Language: English.

L9 ANSWER 23 OF 62 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2001:220695 Document No.: PREV200100220695. Nasal administration of admixed E. coli heat-labile toxin B subunit (**EtxB**) and insulin prevents autoimmune diabetes melitus (iDDM) in NOD mice by inducing regulatory CD4+ cells. **Williams, Neil A.** [Reprint author]; Turcanu, Victor [Reprint author]. Department of Pathology and Microbiology, University of Bristol, University Walk, Bristol, BS8 1TD, UK. Diabetes-Metabolism Research and Reviews, (January-February, 2001) Vol. 17, No. Suppl. 1, pp. S36. print.
Meeting Info.: 5th International Congress of the Immunology of Diabetes Society. Madras, Chennai, India. February 13-16, 2001. Immunology of Diabetes Society.
ISSN: 1520-7552. Language: English.

L9 ANSWER 24 OF 62 MEDLINE on STN DUPLICATE 21

20012221761. PubMed ID: 11111925. Immune modulation by the cholera-like enterotoxin B-subunits: from adjuvant to immunotherapeutic. **Williams N A**. (University of Bristol, Department of Pathology and Microbiology, School of Medical Sciences, UK.. Neil.a.williams@bris.ac.uk) . International journal of medical microbiology : IJMM, (2000 Oct) 290 (4-5) 447-53. Ref: 43. Journal code: 100898849. ISSN: 1438-4221. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Cholera toxin (Ctx) and its close relative, Escherichia coli heat-labile enterotoxin (Etx) have long been established as potent mucosal and systemic adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here we describe findings which demonstrate that contrary to the established dogma the non-toxic B-subunit of Etx (**EtxB**) is a highly potent mucosal adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling

processes in lymphocyte populations which modulate differentially their activation, differentiation and survival. The elucidation of these properties has led to the further use of **EtxB** as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.

L9 ANSWER 25 OF 62 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2001:82983 Document No.: PREV200100082983. Nasal administration of admixed E. coli heat-labile toxin B subunit (**EtxB**) and insulin prevents autoimmune diabetes mellitus (IDDM) in NOD mice by inducing regulatory CD4+ cells. Turcanu, Victor [Reprint author]; **Williams, Neil A.** [Reprint author]. Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD, UK. Immunology, (December, 2000) Vol. 101, No. Supplement 1, pp. 59. print.
Meeting Info.: Annual Congress of the British Society for Immunology. Harrogate, UK. December 05-08, 2000. British Society for Immunology.
CODEN: IMMUAM. ISSN: 0019-2805. Language: English.

L9 ANSWER 26 OF 62 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

2001:82982 Document No.: PREV200100082982. Immunomodulation of the human MLR by E. coli-heat-labile toxin B subunit (**EtxB**): Induction of regulatory T cells. Turcanu, Victor [Reprint author]; **Williams, Neil A.** [Reprint author]. Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD, UK. Immunology, (December, 2000) Vol. 101, No. Supplement 1, pp. 59. print.
Meeting Info.: Annual Congress of the British Society for Immunology. Harrogate, UK. December 05-08, 2000. British Society for Immunology.
CODEN: IMMUAM. ISSN: 0019-2805. Language: English.

L9 ANSWER 27 OF 62 CAPLUS COPYRIGHT 2004 ACS on STN

1999:736498 Document No. 131:335799 Immunomodulatory activity of B subunits of cholera toxin, verotoxin, and heat-labile enterotoxin. **Hirst, Timothy Raymond; Williams, Neil Andrew** (University of Bristol, UK). PCT Int. Appl. WO 9958145 A2 19991118, 63 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1461 19990510. PRIORITY: GB 1998-9958 19980508; GB 1998-11954 19980603; GB 1998-12316 19980608.

AB The authors disclose the use of: (i) heat-labile enterotoxin B subunit (**EtxB**), cholera toxin B subunit (CtxB) or verotoxin B subunit (VtxB) in vaccine prepns. to alter the immune response to pathogens. In one example, the secretory IgA response to herpes virus glycoproteins is enhanced by the adjuvant activity of **EtxB**. In addition, the authors disclose the use of agents other than **EtxB** or CtxB, which have ganglioside GM1-binding activity, or an agent other than VtxB which has globotriosylceramide (Gb3)-binding activity for affecting intracellular signaling events.

L9 ANSWER 28 OF 62 CAPLUS COPYRIGHT 2004 ACS on STN

1999:451202 Document No. 131:82960 **EtxB** or ganglioside GM1 for treating allergic or hypersensitivity conditions. **Williams, Neil Andrew; Hirst, Timothy Raymond; Bienenstock, John** (Oratol Limited, UK). PCT Int. Appl. WO 9934817 A1 19990715, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW,

MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB70 19990108. PRIORITY: GB 1998-487 19980109.

AB The use of an agent in the manufacture of a medicament to treat an allergic condition and/or a hypersensitivity condition is described. The agent is capable of modulating a ganglioside-associated activity. The agent is not coupled to an antigen. The modulation of the ganglioside-associated activity affects an allergic condition and/or a hypersensitivity condition. Examples of such modulators include ganglioside GM1 and E. coli enterotoxin B subunit.

L9 ANSWER 29 OF 62 MEDLINE on STN DUPLICATE 22

1999238511. PubMed ID: 10220447. Intranuclear delivery of an antiviral peptide mediated by the B subunit of Escherichia coli heat-labile enterotoxin. Loregian A; Papini E; Satin B; Marsden H S; **Hirst T R** ; Palu G. (Institute of Microbiology, University of Padua, 35121 Padua, Italy.) Proceedings of the National Academy of Sciences of the United States of America, (1999 Apr 27) 96 (9) 5221-6. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We report an intracellular peptide delivery system capable of targeting specific cellular compartments. In the model system we constructed a chimeric protein consisting of the nontoxic B subunit of Escherichia coli heat-labile enterotoxin (**EtxB**) fused to a 27-mer peptide derived from the DNA polymerase of herpes simplex virus 1. Viral DNA synthesis takes places in the nucleus and requires the interaction with an accessory factor, UL42, encoded by the virus. The peptide, designated Pol, is able to dissociate this interaction. The chimeric protein, **EtxB**-Pol, retained the functional properties of both **EtxB** and peptide components and was shown to inhibit viral DNA polymerase activity in vitro via disruption of the polymerase-UL42 complex. When added to virally infected cells, **EtxB**-Pol had no effect on adenovirus replication but specifically interfered with herpes simplex virus 1 replication. Further studies showed that the antiviral peptide localized in the nucleus, whereas the **EtxB** component remained associated with vesicular compartments. The results indicate that the chimeric protein entered through endosomal acidic compartments and that the Pol peptide was cleaved from the chimeric protein before being translocated into the nucleus. The system we describe is suitable for delivery of peptides that specifically disrupt protein-protein interactions and may be developed to target specific cellular compartments.

L9 ANSWER 30 OF 62 MEDLINE on STN DUPLICATE 23

1999134317. PubMed ID: 9933586. Structural basis for the differential toxicity of cholera toxin and Escherichia coli heat-labile enterotoxin. Construction of hybrid toxins identifies the A2-domain as the determinant of differential toxicity. Rodighiero C; Aman A T; Kenny M J; Moss J; Lencer W I; **Hirst T R**. (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, United Kingdom.) Journal of biological chemistry, (1999 Feb 12) 274 (7) 3962-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cholera toxin (Ctx) and E. coli heat-labile enterotoxin (Etx) are structurally and functionally similar AB5 toxins with over 80% sequence identity. When their action in polarized human epithelial (T84) cells was monitored by measuring toxin-induced Cl- ion secretion, Ctx was found to be the more potent of the two toxins. Here, we examine the structural basis for this difference in toxicity by engineering a set of mutant and hybrid toxins and testing their activity in T84 cells. This revealed that the differential toxicity of Ctx and Etx was (i) not due to differences in the A-subunit's C-terminal KDEL targeting motif (which is RDEL in Etx), as a KDEL to RDEL substitution had no effect on cholera toxin activity; (ii) not attributable to the enzymatically active A1-fragment, as hybrid toxins

in which the A1-fragment in Ctx was substituted for that of Etx (and vice versa) did not alter relative toxicity; and (iii) not due to the B-subunit, as the replacement of the B-subunit in Ctx for that of Etx caused no alteration in toxicity, thus excluding the possibility that the broader receptor specificity of **EtxB** is responsible for reduced activity. Remarkably, the difference in toxicity could be mapped to a 10-amino acid segment of the A2-fragment that penetrates the central pore of the B-subunit pentamer. A comparison of the in vitro stability of two hybrid toxins, differing only in this 10-amino acid segment, revealed that the Ctx A2-segment conferred a greater stability to the interaction between the A- and B-subunits than the corresponding segment from Etx A2. This suggests that the reason for the relative potency of Ctx compared with Etx stems from the increased ability of the A2-fragment of Ctx to maintain holotoxin stability during uptake and transport into intestinal epithelia.

L9 ANSWER 31 OF 62 CAPLUS COPYRIGHT 2004 ACS on STN
2000:883742 Document No. 135:44842 Immune modulation by the cholera-like enterotoxin B-subunits: From adjuvant to immunotherapeutic. Pitman, Richard S.; **Hirst, Timothy R.**; **Williams, Neil A.** (Division of Gastroenterology, Department of Medicine, Brigham and Women's Hospital, Boston, MA, 02115, USA). Recent Research Developments in Immunology, 1(Pt. 2), 497-511 (English) 1999. CODEN: RRDIB8. Publisher: Research Signpost.
AB A review with 59 refs. Cholera toxin (Ctx) and its close relative, Escherichia coli heat-labile enterotoxin (Etx) have long been established as potent mucosal and systemic adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here the authors describe findings which demonstrate that the non-toxic B-subunit of Etx (**EtxB**) is a highly potent mucosal adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their activation, differentiation, and survival. The elucidation of these properties has led to the further use of **EtxB** as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.

L9 ANSWER 32 OF 62 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
2000:155508 Document No.: PREV200000155508. Signalling events induced by E. coli heat-labile enterotoxin B-subunit-receptor binding. Bone, Heather K. [Reprint author]; Pitman, Richard S. [Reprint author]; **Williams, Neil A.** [Reprint author]. Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD, UK. Immunology, (Dec., 1999) Vol. 98, No. suppl. 1, pp. 174. print.
Meeting Info.: Joint Congress of the British Society for Immunology and the British Society for Allergy and Clinical Immunology. Harrogate, England, UK. November 30-December 03, 1999. British Society for Allergy and Clinical Immunology; British Society for Immunology.
CODEN: IMMUAM. ISSN: 0019-2805. Language: English.

L9 ANSWER 33 OF 62 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
2000:138014 Document No.: PREV200000138014. **EtxB** induces IL-10 production by human monocytes and has immunomodulating effects upon the mixed lymphocyte reaction. Turcanu, V. [Reprint author]; Heaton, C. P. E. [Reprint author]; **Williams, N. A.** [Reprint author]. Department of Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD, UK. Immunology, (Dec., 1999) Vol. 98, No. suppl. 1, pp. 36. print.
Meeting Info.: Joint Congress of the British Society for Immunology and the British Society for Allergy and Clinical Immunology. Harrogate, England, UK. November 30-December 03, 1999. British Society for Allergy

and Clinical Immunology; British Society for Immunology. CODEN: IMMUAM. ISSN: 0019-2805. Language: English.

L9 ANSWER 34 OF 62 CAPLUS COPYRIGHT 2004 ACS on STN
1999:36021 Document No. 130:164241 Receptor mediated apoptosis of CD8+T cells by the B subunits of cholera-like enterotoxins. Pitman, Richard S.; **Hirst, Timothy R.**; Nashar, Toufic O.; **Williams, Neil A.** (Department of Pathology and Microbilogy, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK). Biochemical Society Transactions, 26(4), S338 (English) 1998. CODEN: BCSTB5. ISSN: 0300-5127. Publisher: Portland Press Ltd..

AB Heat-labile enterotoxin (Etx) B subunit (**EtxB**) and cholera toxin (Ctx) B subunit directly mediate apoptosis of CD8+T cells through an interaction with GM1, present on lymphocyte cell surfaces. Although the precise signaling pathways which mediate **EtxB** induced cellular activation and apoptosis remain unknown, it has been demonstrated that resp. levels of ceramide and MAPK (mitogen-activated protein kinase) activity remain unaltered in both T and B lymphocytes upon addition of **EtxB**, thereby excluding a role for these signaling mechanisms.

L9 ANSWER 35 OF 62 MEDLINE on STN DUPLICATE 24
97289759. PubMed ID: 9144230. Prevention of autoimmune disease due to lymphocyte modulation by the B-subunit of Escherichia coli heat-labile enterotoxin. **Williams N A**; Stasiuk L M; Nashar T O; Richards C M; Lang A K; Day M J; **Hirst T R**. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom.) Proceedings of the National Academy of Sciences of the United States of America, (1997 May 13) 94 (10) 5290-5. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We demonstrate that the receptor binding moiety of Escherichia coli heat-labile enterotoxin (**EtxB**) can completely prevent autoimmune disease in a murine model of arthritis. Injection of male DBA/1 mice at the base of the tail with type II collagen in the presence of complete Freund's adjuvant normally leads to arthritis, as evidenced by inflammatory infiltration and swelling of the joints. A separate injection of **EtxB** at the same time as collagen challenge prevented leukocyte infiltration, synovial hyperplasia, and degeneration of the articular cartilage and reduced clinical symptoms of disease by 82%. The principle biological property of **EtxB** is its ability to bind to the ubiquitous cell surface receptor GM1 ganglioside, and to other galactose-containing glycolipids and galactoproteins. The importance of receptor interaction in mediating protection from arthritis was demonstrated by the failure of a non-receptor-binding mutant of **EtxB** to elicit any protective effect. Analysis of T cell responses to collagen, in cultures of draining lymph node cells, revealed that protection was associated with a marked increase in interleukin 4 production concomitant with a reduction in interferon gamma levels. Furthermore, in protected mice there was a significant reduction in anti-collagen antibody levels as well as an increase in the IgG1/IgG2a ratio. These observations show that protection is associated with a shift in the Th1/Th2 balance as well as a general reduction in the extent of the anti-type II collagen immune response. This suggests that **EtxB** -receptor-mediated modulation of lymphocyte responses provides a means of preventing autoimmune disease.

L9 ANSWER 36 OF 62 MEDLINE on STN DUPLICATE 25
1998018503. PubMed ID: 9378497. Modulation of B-cell activation by the B subunit of Escherichia coli enterotoxin: receptor interaction up-regulates MHC class II, B7, CD40, CD25 and ICAM-1. Nashar T O; **Hirst T R**; **Williams N A**. (School of Medical Sciences, University of Bristol, UK.) Immunology, (1997 Aug) 91 (4) 572-8. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The B subunits of cholera toxin (CtxB) and Escherichia coli heat-labile

enterotoxin (**EtxB**) are non-toxic lectins that bind and cross-link a ubiquitous cell glycolipid receptor, ganglioside GM1, and are recognized as potent mucosal and systemic immunogens. Here we examine the role of **EtxB** receptor occupancy in modulating the activation of B cells, in vitro, in primary lymphocyte cultures containing B and T cells. When 48-hr spleen cell cultures containing **EtxB** were compared with those in the presence of a non-receptor binding mutant, **EtxB**(G33D), a marked shift in the ratio of CD4+ T cells: B cells was noted. Evidence suggested that this was the result of either enhanced survival or proliferation of B cells associated with receptor occupancy by **EtxB**. Investigation revealed that **EtxB** induced only a minimal increase in proliferation above that of **EtxB**(G33D), in mixed cell cultures, and failed to induce any cell division of purified B cells or T cells. In contrast, receptor-binding by **EtxB** markedly up-regulated the expression of major histocompatability complex (MHC) class II, B7, intracellular adhesion molecule-1 (ICAM-1), CD40 and CD25 on the B-cell surface. These results indicate that the polyclonal effects of **EtxB** on B cells are not associated with wide-scale proliferation, but more likely with maintenance of B-cell survival by activation of molecules essential for B-cell differentiation. The findings also highlight the essential role of GM1-interaction with **EtxB** in the regulation of lymphocyte responses.

L9 ANSWER 37 OF 62 MEDLINE on STN DUPLICATE 26

97444582. PubMed ID: 9299772. Hollow-fire bioreactors compared to batch and chemostat culture for the production of a recombinant toxoid by a marine Iibrio. Lloyd J R; **Hirst T R**; Bunch A W. (School of Biological Sciences, University of Birmingham, Edgbaston,. UK.J.R.Lloyd@bham.ac.uk) . Applied microbiology and biotechnology, (1997 Aug) 48 (2) 155-61. Journal code: 8406612. ISSN: 0175-7598. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Bioreactor selection is important for maximising the productivity of recombinant organisms. In this paper a comparison is made between growth and recombinant protein synthesis in three types of bioreactor containing a marine Vibrio capable of heterologous expression and secretion of the non-toxic B-subunit pentamer of Escherichia coli heat-labile enterotoxin, **EtxB**. The heterologous gene was located on the plasmid pMMB68. Resistance to carbenicillin was used to select for plasmid-containing cells. In batch and continuous culture. Volumetric productivities were highest when cells were grown in the presence of carbenicillin. Without antibiotic selection, the highest volumetric productivity (9.4 mg **EtxB**-1 h-1) was observed in hollow-fibre bioreactors, and the production phase could be maintained for over 50 h. The highest specific productivity under these conditions was found in batch culture, but the maximal production phase was only of 5 h duration. In hollow-fibre reactors the type of fibre used significantly affected productivity, both with regards to the maintenance of reactor integrity and by allowing passage of the recombinant toxoid through the selectively permeable membrane. Where contamination of the product with carbenicillin is to be avoided, these bioreactors are superior to batch or continuous culture.

L9 ANSWER 38 OF 62 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 27

97248420 EMBASE Document No.: 1997248420. EDTA protects E. coli heat-labile enterotoxin B subunit-based fusion proteins from proteolytic degradation during their production by Vibrio spp. Loregian A.; Marcello A.; **Hirst T.R.**; Palu G.. G. Palu, Institute of Microbiology, University of Padova, Via A. Gabelli 63, 35121 Padova, Italy. Minerva Biotecnologica 9/2 (61-67) 1997.
Refs: 35.
ISSN: 1120-4826. CODEN: MIBIFK. Pub. Country: Italy. Language: English. Summary Language: English.

AB E. coli heat-labile enterotoxin B subunit (**EtxB**) has been proposed as a potential protein carrier for the delivery of heterologous

peptides into target cells. To thoroughly exploit the biotechnological potential of **EtxB**- based fusion proteins, a simple method has to be worked out for their expression and purification. Production of these chimeric toxins faces problems with regard to both their low yield and stability. Methods. In this study we describe a protocol for the optimal production and secretion of undegraded **EtxB** hybrids into the extracellular medium of cultures of non- toxinogenic Vibrio strains. Results. The highest level of expression of two chimeric toxins, **EtxB**-R2 and **EtxB**-pol, was obtained by using Vibrio sp. 60 cultures, reaching 52 mg/l and 8 mg/l, respectively. The presence of 0.3 mM EDTA in the culture medium totally preserved both chimeric toxins from proteolysis, also during a prolonged expression. Conclusions. This system may be useful for the preparation of other **EtxB**-based fusion proteins.

L9 ANSWER 39 OF 62 MEDLINE on STN DUPLICATE 28

96325012. PubMed ID: 8702586. Assembly of the B subunit pentamer of Escherichia coli heat-labile enterotoxin. Kinetics and molecular basis of rate-limiting steps in vitro. Ruddock L W; Coen J J; Cheesman C; Freedman R B; **Hirst T R**. (Research School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, United Kingdom.) Journal of biological chemistry, (1996 Aug 9) 271 (32) 19118-23. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The B subunits of Escherichia coli heat-labile enterotoxin (**EtxB**) and cholera toxin (CtxB) assemble in vivo into exceptionally stable homopentameric complexes, which maintain their quaternary structure in a range of conditions that would normally be expected to cause protein denaturation. Recently, we showed that the simultaneous protonation of two of the COOH-terminal carboxylates in pentameric **EtxB** was required to cause its disassembly at pH values below 2.0 (Ruddock, L., Ruston, S. P., Kelly, S. M., Price, N. C., Freedman, R. B., and Hirst, T. R.(1995) J. Biol. Chemical 270, 29953-29958). Here, we investigate the influence of environmental parameters on the kinetics of reassembly of acid-generated **EtxB** monomers in vitro. Such monomers were found to undergo a further acid-mediated conformational change, with an activation energy of 76 +/- 2 J.mol-1.K-1, consistent with isomerization of the cis-proline residue at position 93, and which prevented subsequent **EtxB** reassembly. By using rapid neutralization of acid-generated monomers, a high proportion of the B-subunits adopted an assembly-competent conformation, which resulted in up to 75% of the protein reassembling into a stable pentameric complex, indistinguishable from native **EtxB** pentamers. The rate-limiting step in reassembly, over a concentration range of 50-200 microg/ml, was shown to be due to an intramolecular event, which exhibited a pH dependence with a pKa of 7.0. Modification of **EtxB** with amine-specific probes revealed that the protonation state of the NH2-terminal alanine residue was responsible for the pH dependence of reassembly. The implications of these findings for the biogenesis of Escherichia coli enterotoxin and related enterotoxins in vivo, are considered.

L9 ANSWER 40 OF 62 MEDLINE on STN DUPLICATE 29

97128619. PubMed ID: 8973177. A pH-dependent conformational change in the B-subunit pentamer of Escherichia coli heat-labile enterotoxin: structural basis and possible functional role for a conserved feature of the AB5 toxin family. Ruddock L W; Webb H M; Ruston S P; Cheesman C; Freedman R B; **Hirst T R**. (Research School of Biosciences, University of Kent at Canterbury, U.K.. l.w.ruddock@ukc.ac.uk) . Biochemistry, (1996 Dec 17) 35 (50) 16069-76. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The non-covalently associated B-subunit moieties of AB5 toxins, such as cholera toxin and related diarrheagenic enterotoxins, exhibit exceptional pH stability and remain pentameric at pH values as low as 2.0. Here, we investigate the structural basis of a pH-dependent conformational change which occurs within the B5 structure of Escherichia coli heat-labile

enterotoxin (**EtxB**) at around pH 5.0. The use of far-UV CD and fluorescence spectroscopy showed that **EtxB** pentamers undergo a fully reversible pH-dependent conformational change with a pKa of 4.9 +/- 0.1 (R2 = 0.999) or 5.13 +/- 0.01 (R2 = 0.999), respectively. This renders the pentamer susceptible to SDS-mediated disassembly and decreases its thermal stability by 18 degrees C. A comparison of the pH-dependence of the structural change in EtxB5, with that of a mutant containing a Ser substitution at His 57, revealed that the pKa of the conformational change was shifted from ca. 5.1 to 4.4. This finding suggests that protonation of the imidazole side chain of His 57 might facilitate disruption of a spatially adjacent salt bridge, located between Glu 51 and Lys 91 in each B-subunit, thus triggering the conformational change in the pentameric structure. The pH-dependent conformational change was found to be inhibited when B-subunits bound to monosialoganglioside, GMI; and to have no effect on the stability of interaction between A- and B-subunits within the AB5 complex. This suggests that the conformational change is unlikely to have a direct involvement in toxicity. Conservation of the pH-dependent conformational change in the AB5 toxin family, combined with the potential exposure of the hydrophobic core of beta-barrel in the monomeric units, leads to the proposal that the conformational change may be the common feature that ensures the secretion of these proteins from the Vibrionaceae.

L9 ANSWER 41 OF 62 MEDLINE on STN DUPLICATE 30

96324796. PubMed ID: 8671661. Cross-linking of cell surface ganglioside GM1 induces the selective apoptosis of mature CD8+ T lymphocytes. Nashar T O; **Williams N A**; **Hirst T R**; Nahar T O. (Research School of Biosciences, University of Kent, Canterbury, UK.) International immunology, (1996 May) 8 (5) 731-6. Ref: 24. Journal code: 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Gangliosides are glycosphingolipids found ubiquitously on the surface of mammalian cells. They contain a ceramide tail that is inserted into the membrane and exposed carbohydrate and sialic acid moieties. The non-toxic B subunit oligomer (**EtxB**) of Escherichia coli heat-labile enterotoxin (Etx) is a potent immunogen in vivo and has profound modulatory effects on **EtxB**-primed lymphocytes in vitro, properties which are dependent on its ability to bind to GM1 ganglioside receptors. Here, it is shown that cross-linking GM1 by **EtxB** causes a differential effect on mature CD4(+) and CD8(+) T cells from lymph node cultures proliferating in response to an unrelated antigen, ovalbumin. Addition of **EtxB** to such cultures led to the complete depletion of CD8(+) T cells compared with enhanced activation of CD4(+) cells [as measured by expression of CD25 (IL-2Ralpha)]. By contrast, addition of a mutant **EtxB**, **EtxB**(G33D), which does not bind to GM1, failed to trigger CD8(+) T cell depletion. When **EtxB** was added to isolated non-immune CD8(+) lymphocytes rapid (12-18 h) alterations in nuclear morphology and the appearance of sub-G0/G1 levels of DNA were induced; properties which are characteristic of cells undergoing apoptosis. **EtxB**(G33D) failed to trigger apoptosis, indicating that the induction of the apoptotic signal was dependent on the binding of GM1. These findings provide an insight into the potent immunogenicity and immunomodulatory properties of E. coli enterotoxins as well as heralding a novel method for the selective induction of apoptosis in mature CD8(+) T lymphocytes.

L9 ANSWER 42 OF 62 MEDLINE on STN DUPLICATE 31

97090693. PubMed ID: 8936601. Use of Vibrio spp. for expression of Escherichia coli enterotoxin B subunit fusion proteins: purification and characterization of a chimera containing a C-terminal fragment of DNA polymerase from herpes simplex virus type 1. Loregian A; **Hirst T R** ; Marsden H S; Palu G. (Institute of Microbiology, University of Padova, Italy.) Protein expression and purification, (1996 Nov) 8 (3) 381-9. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB The nontoxic B subunit of Escherichia coli heat-labile enterotoxin (**EtxB**) is a convenient carrier molecule for the attachment and delivery of heterologous peptides into eukaryotic cells. To evaluate the properties of such **EtxB**-based fusion proteins an efficient method for their production and purification is required. High-level production and purification of native **EtxB** has been achieved using heterologous expression and secretion in a marine Vibrio (Amin, T., and Hirst, T. R., 1994, Protein Expression Purif. 5, 198-204). However, the use of this method to isolate **EtxB** fusion proteins has been precluded because of their susceptibility to degradation by extracellular proteases secreted by members of the Vibrionaceae. In this paper a method is described for production of **EtxB**-pol, comprising the enterotoxin B subunit linked to a 27-residue C-terminal fragment of Pol, the catalytic subunit of DNA polymerase of herpes simplex virus type 1 (HSV-1). Following assessment of the relative efficacy of different Vibrio strains as hosts for **EtxB**-pol expression, the chimera was produced at the highest level of 3.5 mg/liter by cultures of Vibrio sp.60. Addition of 0.3 mM EDTA to the growth medium blocked proteolysis of the secreted **EtxB**-pol fusion protein, which was then purified to homogeneity using ammonium sulfate fractionation and hydrophobic interaction chromatography, with a yield of 57%. Purified **EtxB** -pol reacted with both anti-**EtxB** and anti-Pol peptide antibodies, and was able to specifically bind UL42, a processivity factor which normally binds to the C-terminal region of HSV-1 Pol. This modified method for expression and purification of **EtxB**-pol should be of general utility for the preparation of other **EtxB**-based fusion proteins.

L9 ANSWER 43 OF 62 MEDLINE on STN DUPLICATE 32

96133910. PubMed ID: 8552610. Potent immunogenicity of the B subunits of Escherichia coli heat-labile enterotoxin: receptor binding is essential and induces differential modulation of lymphocyte subsets. Nashar T O; Webb H M; Eaglestone S; **Williams N A**; **Hirst T R**. (Research School of Biosciences, University of Kent, Canterbury, Great Britain.) Proceedings of the National Academy of Sciences of the United States of America, (1996 Jan 9) 93 (1) 226-30. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The importance of receptor binding in the potent immunogenicity of Escherichia coli heat-labile enterotoxin B subunit (**EtxB**) was tested by comparing its immunogical properties with those of a receptor binding mutant, **EtxB**(G33D). Subcutaneous immunization of **EtxB**(G33D) resulted in 160-fold reduction in antibody titer compared with wild-type **EtxB**, whereas its oral delivery failed to provoke any detectable secretory or serum anti-B subunit responses. Moreover, the two proteins induced strikingly different effects on lymphocyte cultures in vitro. **EtxB**, in comparison with **EtxB**(G33D), caused an increase in the proportion of B cells, many of which were activated (CD25+); the complete depletion of CD8+ T cells; an increase in the activation of CD4+ T cells; and an increase in interleukin 2 and a decrease in interferon gamma. These data indicate that **EtxB** exerts profound effects on immune cells, suggesting that its potent immunogenicity is dependent not only on efficient receptor-mediated uptake, but also on direct receptor-mediated immunomodulation of lymphocyte subsets.

L9 ANSWER 44 OF 62 MEDLINE on STN DUPLICATE 33

96102052. PubMed ID: 8530395. Kinetics of acid-mediated disassembly of the B subunit pentamer of Escherichia coli heat-labile enterotoxin. Molecular basis of pH stability. Ruddock L W; Ruston S P; Kelly S M; Price N C; Freedman R B; **Hirst T R**. (Biological Laboratory, University of Kent, Canterbury, United Kingdom.) Journal of biological chemistry, (1995 Dec 15) 270 (50) 29953-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The B-subunit pentamer of Escherichia coli heat-labile enterotoxin (

EtxB) is highly stable, maintaining its quaternary structure in a range of conditions that would normally be expected to cause protein denaturation. In this paper the structural stability of **EtxB** has been studied as a function of pH by electrophoretic, immunochemical, and spectroscopic techniques. Disassembly of the cyclic pentameric structure of human **EtxB** occurs only below pH 2. As determined by changes in intrinsic fluorescence this process follows first-order kinetics, with the rate constant for disassembly being proportional to the square of the H+ ion concentration, and with an activation energy of 155 kJ mol-1. A C-terminal deletion mutant, hEtxB214, similarly shows first-order kinetics for disassembly but with a higher pH threshold, resulting in disassembly being seen at pH 3.4 and below. These findings are consistent with the rate-limiting step for disassembly of human **EtxB** being the simultaneous disruption of two interfaces by protonation of two C-terminal carboxylates. By comparison, disassembly of the B-subunit of cholera toxin (CtxB), a protein which shows 80% sequence identity with **EtxB**, exhibits a much lower stability to acid conditions; with disassembly of CtxB occurring below pH 3.9, with an activation energy of 81 kJ mol-1. Reasons for the observed differences in acid stability are discussed, and the implications of these findings to the development of oral vaccines using **EtxB** and CtxB are considered.

L9 ANSWER 45 OF 62 MEDLINE on STN DUPLICATE 34

95378272. PubMed ID: 7544352. Generation of a monoclonal antibody that recognizes the amino-terminal decapeptide of the B-subunit of Escherichia coli heat-labile enterotoxin. A new probe for studying toxin assembly intermediates. Amin T; Larkins A; James R F; **Hirst T R**. (Research School of Biosciences, University of Kent, Canterbury, United Kingdom.) Journal of biological chemistry, (1995 Aug 25) 270 (34) 20143-50. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cholera toxin and the related Escherichia coli heat-labile enterotoxin are hexameric proteins comprising one A-subunit and five B-subunits. In this paper we report the generation and characterization of a monoclonal antibody, designated LDS47, that recognizes and precipitates in vivo assembly intermediates of the B-subunit (**EtxB**) of E. coli heat-labile enterotoxin. The monoclonal antibody is unable to precipitate native B-subunit pentamers, thus making LDS47 a useful probe for studying the early stages of enterotoxin biogenesis. The use of LDS47 to monitor the in vivo turnover of newly synthesized B-subunits in the periplasm of E. coli demonstrated that (i) the turnover of unassembled B-subunits followed an apparent first order process and (ii) it occurred concomitantly with the assembly of native B-pentamers ($k = 0.317 +/- 0.170$ min-1; $t1/2 = 2.2$ min). No other proteins were co-precipitated with the newly synthesized B-subunits; a finding that implies that unassembled B-subunits do not stably associate with other periplasmic proteins prior to their assembly into a macromolecular complex. The use of overlapping synthetic peptides corresponding to the entire **EtxB** polypeptide demonstrated that the epitope recognized by LDS47 is located within the amino-terminal decapeptide of the B-subunit. From the x-ray structural analysis of the toxin (Sixma, T., Kalk, K., van Zanten, B., Dauter, Z., Kingma, J., Witholt, B., and Hol, W. G. J. (1993) J. Mol. Biol. 230, 890-918), this region appears to resemble a curved finger that clasps the adjacent B-subunit. Thus, this region might be expected to be exposed in the unfolded or unassembled subunit, but to become partially buried upon assembly and thus inaccessible to recognition by the monoclonal antibody.

L9 ANSWER 46 OF 62 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

95:524300 The Genuine Article (R) Number: RL566. IMMUNOREGULATORY ROLE OF H-2 AND INTRA-H-2 ALLELES ON ANTIBODY-RESPONSES TO RECOMBINANT PREPARATIONS OF B-SUBUNITS OF ESCHERICHIA-COLI HEAT-LABILE ENTEROTOXIN (RETXB) AND CHOLERA-TOXIN (RCTXB). NASHAR T O (Reprint); **HIRST T R**. UNIV

KENT, RES SCH BIOSCI, CANTERBURY CT2 7NJ, KENT, ENGLAND (Reprint). VACCINE (JUN 1995) Vol. 13, No. 9, pp. 803-810. ISSN: 0264-410X. Pub. country: ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The immunoregulatory role of H-2 and intra-H-2 alleles on antibody responses to recombinant preparations of B-subunits of Escherichia coli heat-labile enterotoxin (rEtxB) and cholera toxin (rCtxB) is reported. Oral delivery of rEtxB to congenic mice of several different H-2 haplotypes resulted in H-2 dependent serum IgG responses (H-2(d) > H-2(b) = H-2(q) > H-2(a) > H-2(k)) and a similar spectrum of intestinal IgA responses in those strains tested Responses to rEtxB and rCtxB were found to be differentially modulated by the H-2 locus, with significant differential effects in H-2(b) and H-2(d) congenic strains (H-2(d) > H-2(b) for rEtxB; H-2(b) > H-2(d) for rCtxB), Additionally, it was found that when rEtxB was fed to mice previously primed (orally) with either rEtxB or rCtxB only those mice primed with rEtxB exhibited a booster response. A second booster immunisation with rEtxB in rCtxB-primed mice produced an H-2 dependent spectrum of responses characteristic of those elicited by rEtxB, with the antibodies predominantly directed against rEtxB and not rCtxB. These results indicate that the differential response to rEtxB and rCtxB is set at the T- and B-cell level. Also, immunoregulation of antibody responses to rEtxB by intra-H-2 I-E in mice transgenic for the entire IE(a)(k) gene was investigated. No significant difference between responses in transgene-positive and -negative mice was found, suggesting that antigen presentation does not involve I-E, but occurs in the context of I-A. The implications of these results for the design of vaccines against enterotoxigenic E. coli and cholera diarrhoea are discussed.

L9 ANSWER 47 OF 62 CAPLUS COPYRIGHT 2004 ACS on STN
1995:404765 Document No. 122:233725 Construction of a fusion protein between B subunit of E. coli heat-labile enterotoxin and the C-terminus of herpes simplex virus-DNA polymerase. Loregian, Arianna; Marcello, Alessandro; **Hirst, Timothy R.**; Marsden, Howard S.; Palu, Giorgio (Institute of Microbiology, Univ. of Padova, Italy). Biochemical Society Transactions, 23(1), 61S (English) 1995. CODEN: BCSTB5. ISSN: 0300-5127. Publisher: Portland Press.

AB It was recently reported that the B subunit of heat-labile enterotoxin from Escherichia coli (**EtxB**) could be used as a recombinant carrier for the receptor-mediated delivery of a peptide fused to it. This was further examined here by characterizing the fusion protein obtained by genetically linking the C-terminal 27 amino acids of HSV-1 DNA polymerase to the C-terminus of **EtxB** (**EtxB**-DNApol). The novel polypeptide was overexpressed in E. coli XL1-Blue and shown to be translocated to the periplasmic compartment at an approx. 10-fold lower level than wild-type **EtxB** expressed under the same conditions. The same experiment also indicated that **EtxB**-DNApol was properly assembled into pentamers capable of binding GM1.

L9 ANSWER 48 OF 62 MEDLINE on STN
95278544. PubMed ID: 7758771. Strategies for the purification of intact and proteolytically-activated native and engineered heat-labile enterotoxins of Escherichia coli. Ruston S; Eaglestone S; Webb H; **Hirst T R.** (Research School of Biosciences, University of Kent, Canterbury, UK.) Biochemical Society transactions, (1995 Feb) 23 (1) 55S. Journal code: 7506897. ISSN: 0300-5127. Pub. country: ENGLAND: United Kingdom. Language: English.

L9 ANSWER 49 OF 62 CAPLUS COPYRIGHT 2004 ACS on STN
1995:404738 Document No. 122:209427 A pleiotropic secretion mutant of Aeromonas hydrophila is unable to secrete heterologously expressed E. coli enterotoxin: implication for common mechanisms of protein secretion. Yu, Jun; **Hirst, Timothy R.** (Res. Sch. of Biosciences, Univ. of Kent, Canterbury/Kent, CT2 7NJ, UK). Biochemical Society Transactions, 23(1),

34S (English) 1995. CODEN: BCSTB5. ISSN: 0300-5127. Publisher: Portland Press.

AB The exeE gene of A. hydrophila is associated with and operon (exe) previously predicted to encode the secretory machinery for aerolysin. Heterologous expression of the **etxB** gene of Escherichia coli (which encodes a heat-labile enterotoxin subunit) in wild-type and exeE gene insertion mutants of A. hydrophila showed that the wild-type but not the mutant organism secreted **EtxB** protein. The exeE gene of A. hydrophila is a homolog of the epsE gene of Vibrio cholerae. The protein encoded by the latter has significant homol. with the ExeE protein and gene epsE complements V. cholerae secretion mutants. Thus, data from the pleiotropic mutant support a common secretion mechanism for toxins from V. cholerae and A. hydrophila.

L9 ANSWER 50 OF 62 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

1994:479508 Document No.: PREV199497492508. Specific inhibition of herpes virus replication by receptor-mediated entry of an antiviral peptide linked to Escherichia coli enterotoxin B subunit. Marcello, Alessandro; Loregian, Arianna; Cross, Anne; Marsden, Howard; **Hirst, Timothy R.** ; Palu, Giorgio [Reprint author]. Inst. Microbiol., Univ. Padova, 35121 Padova, Italy. Proceedings of the National Academy of Sciences of the United States of America, (1994) Vol. 91, No. 19, pp. 8994-8998. CODEN: PNASA6. ISSN: 0027-8424. Language: English.

AB Mimetic peptides capable of selectively disrupting protein-protein interactions represent potential therapeutic agents for inhibition of viral and cellular enzymes. This approach was first suggested by the observation that the peptide YAGAVVNDL, corresponding to the carboxyl-terminal 9 amino acids of the small subunit of ribonucleotide reductase of herpes simplex virus, specifically inhibited the viral enzyme in vitro. Evaluation and use of this peptide as a potential antiviral agent has, however, been thwarted by its failure to inhibit virus replication in vivo, presumably because the peptide is too large to enter eukaryotic cells unaided. Here, we show that the nontoxic B subunit of Escherichia coli heat-labile enterotoxin can be used as a recombinant carrier for the receptor-mediated delivery of YAGAVVNDL into virally infected cells. The resultant fusion protein specifically inhibited herpes simplex virus type 1 replication and ribonucleotide reductase activity in quiescent Vero cells. Preincubation of the fusion protein with soluble GM1 ganglioside abolished this antiviral effect, indicating that receptor-mediated binding to the target cell is necessary for its activity. This provides direct evidence of the usefulness of carrier-mediated delivery to evaluate the intracellular efficacy of a putative antiviral peptide.

L9 ANSWER 51 OF 62 MEDLINE on STN DUPLICATE 35

94331983. PubMed ID: 8054855. Purification of the B-subunit oligomer of Escherichia coli heat-labile enterotoxin by heterologous expression and secretion in a marine vibrio. Amin T; **Hirst T R.** (Biological Laboratory, The University, Canterbury, Kent, United Kingdom.) Protein expression and purification, (1994 Apr) 5 (2) 198-204. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Heat-labile enterotoxins (Etx) are plasmid-encoded, multimeric proteins produced by certain diarrheagenic strains of Escherichia coli. The nontoxic, receptor-binding B subunit (**EtxB**) of such toxins may be useful as a component of vaccines against enterotoxigenic E. coli, or as a carrier for the delivery of heterologous epitopes to the mucosal immune system. Here we describe a simple method for the purification of **EtxB** from a marine vibrio harboring a broad-host range controlled expression vector containing the **etxB** gene. Induction of **EtxB** resulted in its specific secretion to the medium, to a concentration of greater than 25 mg/liter of culture. The techniques of ultrafiltration and hydrophobic interaction chromatography were used to purify **EtxB** to homogeneity from the medium of this organism

(with a yield of 60.7%). **EtxB**-epitope fusion proteins were also successfully expressed and secreted in this marine vibrio, suggesting that this system may be of general use in the preparation of **EtxB** -based vaccines.

L9 ANSWER 52 OF 62 MEDLINE on STN DUPLICATE 36
94237439. PubMed ID: 8181710. Efficient extracellular production of hybrid E. coli heat-labile enterotoxin B subunits in a marine Vibrio. Marcello A; Loregian A; Palu G; **Hirst T R.** (Institute of Microbiology, University of Padua, Italy.) FEMS microbiology letters, (1994 Mar 15) 117 (1) 47-51. Journal code: 7705721. ISSN: 0378-1097. Pub. country: Netherlands. Language: English.

AB Escherichia coli heat-labile enterotoxin B subunit (**EtxB**) has been proposed as a potential protein carrier for the delivery of heterologous peptides to target cells, particularly for the oral delivery of epitopes to the mucosal immune system. In this study, two extensions to the C-terminus of **EtxB** were genetically engineered that correspond to a well-characterized neutralising epitope of glycoprotein D from herpes simplex virus (**EtxB**-gD) and to the C-terminal nine amino acids from the 38 kDa subunit of HSV-encoded ribonucleotide reductase (**EtxB**-R2). Here we describe the extracellular secretion of the two hybrid **EtxBs** from a marine Vibrio harbouring a broad-host range inducible expression vector containing the hybrid genes. Large amounts of intact fusion proteins (15-20 mg per liter of culture) were secreted into the medium upon induction. These hybrid proteins maintained the receptor-binding activity of the native toxin as well as being cross-reactive with anti-**EtxB** and anti-heterologous peptide monoclonal antibodies.

L9 ANSWER 53 OF 62 MEDLINE on STN DUPLICATE 37
93175126. PubMed ID: 7679865. Current progress in the development of the B subunits of cholera toxin and Escherichia coli heat-labile enterotoxin as carriers for the oral delivery of heterologous antigens and epitopes. Nashar T O; Amin T; Marcello A; **Hirst T R.** (Biological Laboratory, University of Kent, Canterbury, UK.) Vaccine, (1993) 11 (2) 235-40. Ref: 49. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The development of non-living carrier systems for delivery of protective antigens or epitopes to the immune system represents both a fundamental and an applied aspect of vaccinology. A wide range of carrier systems, ranging from inert supports to proteins that exert direct immunomodulating effects on the immune response, are being studied. In this overview we describe the current progress in the development of the B-subunits of cholera toxin and Escherichia coli heat-labile enterotoxin as potential protein carriers for the oral delivery of chemically and genetically attached antigens and epitopes.

L9 ANSWER 54 OF 62 MEDLINE on STN DUPLICATE 38
93365861. PubMed ID: 8359463. A new method for the purification of the B subunit (**EtxB**) of Escherichia coli heat-labile enterotoxin. Amin T; Marcello A; **Hirst T R.** (Biological Laboratory, University of Kent, Canterbury, U.K.) Biochemical Society transactions, (1993 May) 21 (2) 213S. Journal code: 7506897. ISSN: 0300-5127. Pub. country: ENGLAND: United Kingdom. Language: English.

L9 ANSWER 55 OF 62 CAPLUS COPYRIGHT 2004 ACS on STN
1993:424413 Document No. 119:24413 Analysis of enterotoxin synthesis in a Vibrio cholerae strain lacking DsbA, a periplasmic enzyme involved in disulfide bond formation. Findlay, Gordon; Yu, Jun; **Hirst, Timothy R.** (Biol. Lab., Univ. Kent, Canterbury/Kent, CT2 7NJ, UK). Biochemical Society Transactions, 21(2), 212S (English) 1993. CODEN: BCSTB5. ISSN: 0300-5127.

AB To investigate the events of enterotoxin biogenesis the authors used a simplified system consisting of a vibrio strain with a chromosomal ctx

gene deletion harboring the plasmid pMMB107, which encodes only the B-subunit of cholera-like enterotoxin (**EtxB**). Transposon (TnphoA) mutagenesis of this strain resulted in the identification of a mutant, UKC13::TnphoA.7A (pMMB107) with a 50-fold reduction in the level of the **EtxB** secretion. TnphoA insertion was found to be in a gene encoding a periplasmic protein with 40% homol. to the recently identified disulfide bond-forming protein (DsbA) of E. coli. To examine the role of DsbA in ExtB biogenesis, the dsbA::TnphoA mutant strain was cultured in minimal medium, pulse-labeled with 35S-Met and the fate of radiolabeled ExtB in periplasmic and medium fractions analyzed by SDS-PAGE and autoradiog. This demonstrated that **EtxB** was exported to the periplasm in both the mutant and the wild-type strain, but only secreted to the medium in the wild-type strain. The **EtxB** in the periplasm of the mutant strain was rapidly lost, probably as a result of proteolytic degradation This demonstrates that DsbA is not required for translocation of ExtB to the periplasm, but plays an important role in subsequent steps of toxin formation.

L9 ANSWER 56 OF 62 MEDLINE on STN DUPLICATE 39

93101683. PubMed ID: 1465452. Intermolecular interactions between the A and B subunits of heat-labile enterotoxin from Escherichia coli promote holotoxin assembly and stability in vivo. Streatfield S J; Sandkvist M; Sixma T K; Bagdasarian M; Hol W G; **Hirst T R**. (Biological Laboratory, University of Kent, Canterbury, Great Britain.) Proceedings of the National Academy of Sciences of the United States of America, (1992 Dec 15) 89 (24) 12140-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Cholera toxin and the related heat-labile enterotoxin (LT) produced by Escherichia coli consist of a holotoxin of one A subunit and five B subunits (AB5). Here we investigate the domains of the A subunit (EtxA) of E. coli LT which influence the events of B-subunit (**EtxB**) oligomerization and the formation of a stable AB5 holotoxin complex. We show that the C-terminal 14 amino acids of the A subunit comprise two functional domains that differentially affect oligomerization and holotoxin stability. Deletion of the last 14 amino acids (-14) from the A subunit resulted in a molecule that was significantly impaired in its capacity to promote the assembly of a mutant B subunit, EtxB191.5. In contrast, deletion of the last four amino acids (-4) from the A subunit gave a molecule that retained such a capacity. This suggests that C-terminal residues within the -14 to -4 region of the A subunit are important for promoting the oligomerization of **EtxB**. In addition, we demonstrate that the truncated A subunit lacking the last 4 amino acids was unable to form a stable AB5 holotoxin complex even though it promoted B-subunit oligomerization. This suggests that the last 4 residues of the A subunit function as an "anchoring" sequence responsible for maintaining the stability of A/B subunit interaction during holotoxin assembly. These data represent an important example of how intermolecular interactions between polypeptides in vivo can modulate the folding and assembly of a macromolecular complex.

L9 ANSWER 57 OF 62 MEDLINE on STN DUPLICATE 40

92374846. PubMed ID: 1324389. A homologue of the Escherichia coli DsbA protein involved in disulphide bond formation is required for enterotoxin biogenesis in Vibrio cholerae. Yu J; Webb H; **Hirst T R**. (Biological Laboratory, University of Kent, Canterbury, UK.) Molecular microbiology, (1992 Jul) 6 (14) 1949-58. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A strain of Vibrio cholerae, which had been engineered to express high levels of the non-toxic B subunit (**EtxB**) of Escherichia coli heat-labile enterotoxin, was subjected to transposon (TnphoA) mutagenesis. Two chromosomal TnphoA insertion mutations of the strain were isolated that showed a severe defect in the amount of **EtxB** produced. The loci disrupted by TnphoA in the two mutant derivatives were cloned and sequenced, and this revealed that the transposon had inserted at different

sites in the same gene. The open reading frame of the gene predicts a 200-amino-acid exported protein, with a Cys-X-X-Cys motif characteristic of thioredoxin, protein disulphide isomerase, and DsbA (a periplasmic protein required for disulphide bond formation in E. coli). The V. cholerae protein exhibited 40% identity with the DsbA protein of E. coli, including 90% identity in the region of the active-site motif. Introduction of a plasmid encoding E. coli DsbA into the V. cholerae TnphoA derivatives was found to restore enterotoxin formation, whilst expression of Etx or **EtxB** in a dsbA mutant of E. coli confirmed that DsbA is required for enterotoxin formation in E. coli. These results suggest that, since each **EtxB** subunit contains a single intramolecular disulphide bond, a transient intermolecular interaction with DsbA occurs during toxin subunit folding which catalyses formation of the disulphide in vivo.

L9 ANSWER 58 OF 62 MEDLINE on STN DUPLICATE 41

92268852. PubMed ID: 1588306. Expression of the B subunit of Escherichia coli heat-labile enterotoxin in a marine Vibrio and in a mutant that is pleiotropically defective in the secretion of extracellular proteins. Leece R; **Hirst T R**. (Department of Genetics, University of Leicester, UK.) Journal of general microbiology, (1992 Apr) 138 (Pt 4) 719-24. Journal code: 0375371. ISSN: 0022-1287. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A marine Vibrio (designated Vibrio sp. 60) that is related to Vibrio anguillarum was used as a host for a plasmid that encodes the non-toxic B subunit (**EtxB**) of Escherichia coli heat-labile enterotoxin. Expression of **EtxB** in Vibrio sp. 60 resulted in the efficient and selective secretion of the B subunit into the extracellular growth medium. This indicated that Vibrio sp. 60, which does not normally produce cholera-like enterotoxins, nonetheless possesses a secretory machinery that permits these toxins to be translocated across its cytoplasmic and outer membranes. Expression of **EtxB** in a sec mutant of Vibrio sp. 60 (MVT1192), which had previously been shown to be defective in the secretion of several extracellular proteins, resulted in approximately 95% of the B subunit remaining entrapped within the periplasm of the bacterial cell envelope. This implies that the mutation in MVT1192 defines a locus that determines a common step in the secretion of extracellular proteins, including oligomeric toxins.

L9 ANSWER 59 OF 62 MEDLINE on STN DUPLICATE 42

92140031. PubMed ID: 1779757. Targeting and assembly of an oligomeric bacterial enterotoxoid in the endoplasmic reticulum of Saccharomyces cerevisiae. Schonberger O; **Hirst T R**; Pines O. (Department of Molecular Biology, Hebrew University, Hadassah Medical School, Jerusalem, Israel.) Molecular microbiology, (1991 Nov) 5 (11) 2663-71. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A hybrid protein consisting of the Escherichia coli lipoprotein signal sequence attached to the mature sequence of the B subunit of heat-labile enterotoxin (Lipo-**EtxB**) was expressed in yeast and E. coli. Analyses of cell lysates from Saccharomyces cerevisiae and E. coli expressing the protein revealed that both organisms were able to assemble Lipo-**EtxB** into oligomers that were (i) stable in the presence of sodium dodecyl sulphate, (ii) resistant to proteinase K degradation, and (iii) able to bind to GM1-ganglioside receptors. Each of these properties are characteristic of the wild-type B subunit pentamer produced in E. coli. Assembly of Lipo-**EtxB** was found to be unaffected in a sec18 mutant of S. cerevisiae, which possesses a temperature-sensitive defect in protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus, but was found not to assemble in a sec53 mutant, which causes the misfolding of proteins targeted to the ER. A kar2-1 mutation with a defect in the yeast homologue of BiP caused an 18-fold reduction in Lipo-**EtxB** assembly at the non-permissive temperature in S. cerevisiae. However, introduction of the wild-type KAR2 gene on a plasmid

into the kar2-1 mutant completely suppressed the inhibition of Lipo-**EtxB** assembly. This provides the first evidence that KAR2 facilitates the assembly of an oligomeric protein in yeast and thus implicates KAR2 as a 'molecular chaperone'. The possible mechanisms of enterotoxoid assembly in E. coli and S. cerevisiae are discussed.

L9 ANSWER 60 OF 62 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

91:662869 The Genuine Article (R) Number: GT131. TARGETING AND ASSEMBLY OF AN OLIGOMERIC BACTERIAL ENTEROTOXOID IN THE ENDOPLASMIC-RETICULUM OF SACCHAROMYCES-CEREVISIAE. SCHONBERGER O; **HIRST T R**; PINES O (Reprint). HEBREW UNIV JERUSALEM, HADASSAH MED SCH, DEPT MOLEC BIOL, IL-91010 JERUSALEM, ISRAEL; UNIV CANTERBURY, BIOL LAB, CANTERBURY CT2 7NJ, KENT, ENGLAND. MOLECULAR MICROBIOLOGY (1991) Vol. 5, No. 11, pp. 2663-2671 . Pub. country: ISRAEL; ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A hybrid protein consisting of the Escherichia coli lipoprotein signal sequence attached to the mature sequence of the B subunit of heat-labile enterotoxin (Lipo-**EtxB**) was expressed in yeast and E. coli. Analyses of cell lysates from Saccharomyces cerevisiae and E. coli expressing the protein revealed that both organisms were able to assemble Lipo-**EtxB** into oligomers that were (i) stable in the presence of sodium dodecyl sulphate, (ii) resistant to proteinase K degradation, and (iii) able to bind to GM1-ganglioside receptors. Each of these properties are characteristic of the wild-type B subunit pentamer produced in E. coli. Assembly of Lipo-**EtxB** was found to be unaffected in a sec18 mutant of S. cerevisiae, which possesses a temperature-sensitive defect in protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus, but was found not to assemble in a sec53 mutant, which causes the misfolding of proteins targeted to the ER. A kar2-1 mutation with a defect in the yeast homologue of BiP caused an 18-fold reduction in Lipo-**EtxB** assembly at the non-permissive temperature in S. cerevisiae. However, introduction of the wild-type KAR2 gene on a plasmid into the kar2-1 mutant completely suppressed the inhibition of Lipo-**EtxB** assembly. This provides the first evidence that KAR2 facilitates the assembly of an oligomeric protein in yeast and thus implicates KAR2 as a 'molecular chaperone'. The possible mechanisms of enterotoxoid assembly in E. coli and S. cerevisiae are discussed.

L9 ANSWER 61 OF 62 MEDLINE on STN DUPLICATE 43

90368708. PubMed ID: 2203772. Minimal deletion of amino acids from the carboxyl terminus of the B subunit of heat-labile enterotoxin causes defects in its assembly and release from the cytoplasmic membrane of Escherichia coli. Sandkvist M; **Hirst T R**; Bagdasarian M. (Department of Microbiology, Michigan State University, Lansing 48909.) Journal of biological chemistry, (1990 Sep 5) 265 (25) 15239-44. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Minimal alterations at the carboxyl terminus of the B subunit (**EtxB**) of heat-labile enterotoxin from Escherichia coli were found to have a marked effect on the assembly and release of this polypeptide into the periplasm. Nine mutant **EtxB** polypeptides were obtained by genetic manipulation of the 3'-end of the **etxB** gene using Bal31 nuclease digestion and codon substitution. A correlation was observed between the magnitude of the changes introduced at the carboxyl terminus and the extent to which the mutant polypeptides were defective in assembly and release. Some of the mutant B subunits, exemplified by those in which the last 2 amino acids had been deleted or in which the last 4 residues had been replaced by three different ones, were found to be only partially defective, with a proportion being associated with the periplasmic face of the cytoplasmic membrane and the remainder being exported to the periplasm. The portion associated with membranes was detected as monomers on sodium dodecyl sulfate-polyacrylamide gels, whereas the portion exported to the periplasm were detected as assembled

oligomers. We conclude that the last few amino acids at the carboxyl terminus of **EtxB** exert a profound influence on the assembly and release of the B subunit from the cytoplasmic membrane during export in E. coli.

L9 ANSWER 62 OF 62 MEDLINE on STN DUPLICATE 44
88007397. PubMed ID: 2820934. Alterations at the carboxyl terminus change assembly and secretion properties of the B subunit of Escherichia coli heat-labile enterotoxin. Sandkvist M; **Hirst T R**; Bagdasarian M. (Institute for Applied Cell and Molecular Biology, Umea University, Sweden.) Journal of bacteriology, (1987 Oct) 169 (10) 4570-6. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB The gene encoding the B subunit of heat-labile enterotoxin (**etxB**) was mutated at its 3' end by targeted addition of random nucleotide sequences. Gene products from five mutated **etxB** genes, all of which were shown to encode B subunits with short carboxy-terminal amino acid extensions, were analyzed with respect to a range of functional and structural properties. One class of altered B subunits, exemplified by EtxB124 and EtxB138, which both have seven extra amino acid residues, were found to be specifically defective in their ability to stably associate with A subunits and form holotoxin. Other altered B subunits were less subtlely affected by extensions at their C termini and were, in addition to their failure to associate with A subunits, unable to translocate into the periplasm of Escherichia coli, to pentamerize, or to bind to GM1 ganglioside. This suggests that the carboxy-terminal domain of **EtxB** mediates A subunit-B subunit interaction.

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=> d his

     (FILE 'HOME' ENTERED AT 18:13:54 ON 16 DEC 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 18:14:21 ON
     16 DEC 2004
L1          267 S "ETXB"
L2            1 S L1 AND ALLERGY
L3           25 S L1 AND TREATMENT
L4            6 DUP REMOVE L3 (19 DUPLICATES REMOVED)
L5            0 S L1 AND ALLERGEN
L6            0 S L1 AND TYPE I ALLERGIES
L7         9247 S (WILLIAMS N?/AU OR HIRST T?/AU OR BIENENSTOCK J?/AU)
L8          217 S L7 AND ETXB
L9           62 DUP REMOVE L8 (155 DUPLICATES REMOVED)

=> s l9 and conjugate
L10           2 L9 AND CONJUGATE

=> dup remove l10
PROCESSING COMPLETED FOR L10
L11           2 DUP REMOVE L10 (0 DUPLICATES REMOVED)

=> d l11 1-2 cbib abs

L11 ANSWER 1 OF 2 MEDLINE on STN
2004590487. PubMed ID: 15342647. Trafficking of Exogenous Peptides into
     Proteasome-dependent Major Histocompatibility Complex Class I Pathway
     following Enterotoxin B Subunit-mediated Delivery. Hearn Arron R; de Haan
     Lolke; Pemberton Alexander J; Hirst Timothy R; Rivett A
     Jennifer. (Departments of Biochemistry and Pathology and Microbiology,
     School of Medical Sciences, University of Bristol, University Walk,
     Bristol BS8 1TD, United Kingdom. ) Journal of biological chemistry, (2004
     Dec 3) 279 (49) 51315-22. Journal code: 2985121R. ISSN: 0021-9258. Pub.
     country: United States. Language: English.
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AB The B-subunit component of Escherichia coli heat-labile enterotoxin (**EtxB**), which binds to cell surface GM1 ganglioside receptors, was recently shown to be a highly effective vehicle for delivery of conjugated peptides into the major histocompatibility complex (MHC) class I pathway. In this study we have investigated the pathway of epitope delivery. The peptides used contained the epitope either located at the C terminus or with a C-terminal extension. Pretreatment of cells with cholesterol-disrupting agents blocked transport of **EtxB conjugates** to the Golgi/endoplasmic reticulum, but did not affect **EtxB**-mediated MHC class I presentation. Under these conditions, **EtxB conjugates** entered EEA1-positive early endosomes where peptides were cleaved and translocated into the cytosol. Endosome acidification was required for epitope presentation. Purified 20 S immunoproteasomes were able to generate the epitope from peptides in vitro, but 26 S proteasomes were not. Only presentation from the C-terminal extended peptide was proteasome-dependent in cells, and this was found to be significantly slower than presentation from peptides with the epitope at the C terminus. These results implicate the proteasome in the generation of the correct C terminus of the epitope and are consistent with proteasome-independent N-terminal trimming. Epitope presentation was blocked in a TAP-deficient cell line, providing further evidence that conjugated peptides enter the cytosol as well as demonstrating a requirement for the peptide transporter. Our findings demonstrate the utility of **EtxB**-mediated peptide delivery for rapid and efficient loading of MHC class I epitopes in several different cell types. Conjugated peptides are released from early endosomes into the cytosol where they gain access to proteasomes and TAP in the "classical" pathway of class I presentation.

L11 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
1999:736498 Document No. 131:335799 Immunomodulatory activity of B subunits of cholera toxin, verotoxin, and heat-labile enterotoxin. **Hirst, Timothy Raymond**; **Williams, Neil Andrew** (University of Bristol, UK). PCT Int. Appl. WO 9958145 A2 19991118, 63 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1461 19990510. PRIORITY: GB 1998-9958 19980508; GB 1998-11954 19980603; GB 1998-12316 19980608.

AB The authors disclose the use of: (i) heat-labile enterotoxin B subunit (**EtxB**), cholera toxin B subunit (CtxB) or verotoxin B subunit (VtxB) in vaccine prepns. to alter the immune response to pathogens. In one example, the secretory IgA response to herpes virus glycoproteins is enhanced by the adjuvant activity of **EtxB**. In addition, the authors disclose the use of agents other than **EtxB** or CtxB, which have ganglioside GM1-binding activity, or an agent other than VtxB which has globotriosylceramide (Gb3)-binding activity for affecting intracellular signaling events.

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=> s allergy treatment
L12        1267 ALLERGY TREATMENT

=> s l12 and enterotoxin
L13           2 L12 AND ENTEROTOXIN

=> dup remove l13
PROCESSING COMPLETED FOR L13
L14           2 DUP REMOVE L13 (0 DUPLICATES REMOVED)
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